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limit. Increasing the number of records observed thus tends to eliminate errors arising from random uncorrected variations in environment but does not tend to eliminate errors arising from dominance or epistasis or consistently biased and uncorrected environment.

On the other hand, the average record of  $n$  daughters each tested once is correlated with the dam's genotype thus:

$$hgab\sqrt{\frac{n}{1+(n-1)(h^2g^2a^2b^2+e^2r_{EE})}}$$

which approaches

$$\sqrt{\frac{h^2g^2a^2b^2}{h^2g^2a^2b^2+e^2r_{EE}}}$$

as  $n$  becomes indefinitely large. This approaches unity when  $e^2r_{EE}$  is zero. In other words, increasing  $n$  tends to cancel errors arising from the imperfections of both  $h$  and  $g$ , whereas increasing  $m$  tends to cancel the imperfections of  $h_p$  but does nothing to correct for the fact that  $g_p$  is less than unity. However the progeny test involves  $ab$  which arises from the intervening generation of Mendelian segregation and appears not at all in the correlation between dam's record and dam's genotype.

Thus a daughter's record usually is a less accurate (on account of  $ab$ ) indicator of her dam's genotype than the dam's own record is, but the errors in daughters' records tend to be more completely cancelled by the averaging process than is the case with errors in the dam's record. Hence if  $g$  and  $g_p$  were quite small, it *might* be possible for  $n$  to be so large that its cancellation of errors arising from dominance or epistasis would more than make up for the Mendelian sampling errors which affect the progeny average but not the dam's own records.

The problem of the relative accuracy of progeny test and dam's own record as indicators of the dam's genotype therefore becomes the quantitative one of balancing the errors introduced by  $ab$  against the greater effectiveness of the progeny average in eliminating errors introduced by dominance and epistasis. Unfortunately for the simplicity of the answer,  $e^2r_{EE}$  and  $e_p^2r_{E_pE_p}$  cannot safely be considered zero and their existence sets serious limits on the effectiveness of averaging as a means of eliminating errors, either from the daughter average or from the average of the dam's own records.

The value of  $ab$  will be nearly .5 even with moderately large departures from random mating (11, pages 118-119). Substituting that value for  $ab$ , the ratio ( $B$ ) of  $r_{AG}$  to  $r_{MG}$  becomes

$$B = \frac{hg}{h_pg_p} \sqrt{\frac{n}{m}} \sqrt{\frac{1+(m-1)(h_p^2+e_p^2r_{E_pE_p})}{4+(n-1)(h^2g^2+4e^2r_{EE})}} \quad (1)$$

If dam and daughters are produced by the same kind of a breeding system  $g = g_p$  and if dams and daughters are equally typical samples of their generations and if the records used to represent the productiveness of dam and of daughters are chosen by a method equally fair to both,  $h = h_p$  and the first part of this expression cancels. Assuming (as the case most favorable to the progeny test) that  $m = 1$  and squaring to simplify the expression we have

$$B^2 = \frac{n}{4 + (n-1)(h^2g^2 + 4e^2r_{EE})} \quad (2)$$

For the progeny test to be more accurate than the dam's own record  $B^2$  must be larger than 1.0. This condition is satisfied when  $n > 4 + (n-1)(h^2g^2 + 4e^2r_{EE})$ . For this to be true,  $n$  must be larger than 4, even when  $h^2g^2$  and  $e^2r_{EE}$  are extremely small. If  $e^2r_{EE}$  is as large as .25,  $n$  cannot possibly be large enough to make the progeny test average as reliable as the dam's own performance.

Equation 2 is pictured graphically in figures 2 and 3. Since four variables are involved (treating  $h^2g^2$  as a single variable and  $e^2r_{EE}$  as another single variable, since each behaves as such in this statement of the problem) it seems necessary to select certain reasonable or limiting values of one of the four and for this constant value of one variable to show the interrelations of the other three as a curved surface whose height above a base is  $B^2$ .

Figure 2 is designed to show how  $B^2$  varies with  $n$  and with  $h^2g^2$ . The upper surface shows the limiting condition ( $e^2r_{EE} = \text{zero}$ ) most favorable to the progeny test. Such a condition would be encountered in actual practice when half sisters resemble each other only because they have the same dam. Any correlation between half sisters because they are kept under similar environment, or are related through their other parent, or because  $r_{DD}$  or  $r_{II}$  are not zero would have the same kind of effect as giving  $e^2r_{EE}$  a positive value. Some such effects are almost certain to occur in any set of actual data, even when such data have been obtained under experimental conditions controlled as rigidly as possible. The lower surface in figure 2 shows  $B^2$  when  $e^2r_{EE} = .20$ . Such a condition would arise whenever unrelated cows in the same herd showed a correlation of +.20 just because they were managed and tested in the same herd in a population of data coming from many herds kept under varying management. Such a value for  $e^2r_{EE}$  is roughly consistent with much of the C.T.A. and Official data yet analyzed in a way which permits any estimate of  $e^2r_{EE}$ . However the value of  $e^2r_{EE}$  naturally will vary from population to population, since it is a description of the heterogeneity of the conditions under which different sets of half sisters are kept.

The double line in figure 2 shows a plane parallel to the base at the level  $B^2=1.0$ . Points above this level denote conditions under which the progeny test is more accurate than the dam's own record. When  $e^2r_{EE}=0$ , and  $h^2g^2$  is as low as .10, the progeny test surpasses the cow's own record when  $n=5$ , but  $n$  must exceed 6 when  $h^2g^2=.4$  and must exceed 11 when  $h^2g^2=.7$ . This brings out the important points that the progeny test

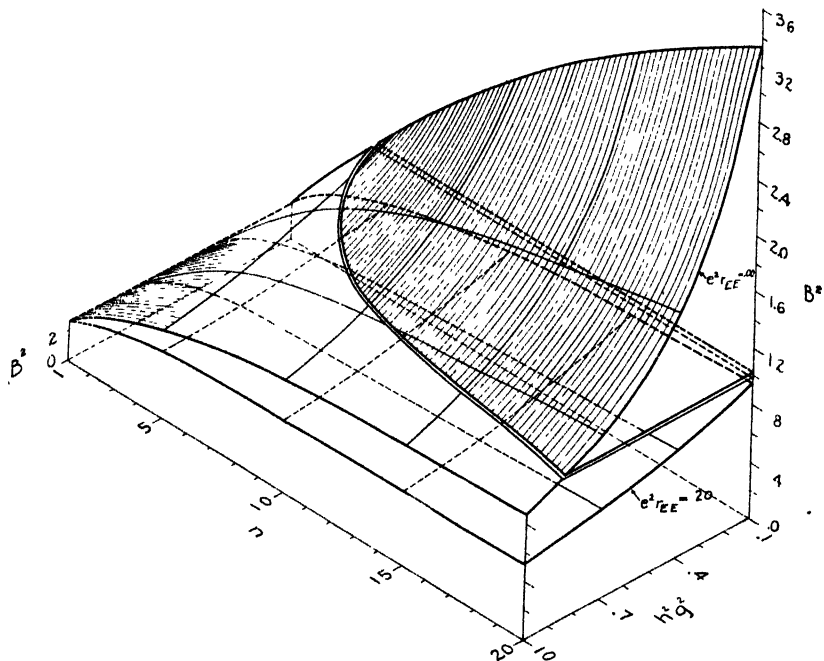


FIG. 2. The relative accuracy of progeny test and the individual's own performance when the number ( $n$ ) of offspring in the progeny test varies and for traits which are highly hereditary ( $h^2g^2$  is large) or slightly hereditary ( $h^2g^2$  is small). The upper surface shows this condition when the offspring resemble each other only because they are half-sibs. The surface at  $e^2r_{EE}=.20$  shows the condition when the offspring resemble each other enough for other reasons to bring about a correlation of +.20 between them on that account alone.

is not very dependable where the daughters resemble each other for other reasons than that they are out of the same dam<sup>1</sup> and that the superiority of the progeny test over the dam's own record is most marked where  $h^2g^2$  is smallest, *i.e.*, where the characteristic is least hereditary in the narrow sense

<sup>1</sup> This same point was shown in more detail by the present writer in an article (8) directed primarily at how  $r_{AG}$  varies with increasing  $n$ . In that article  $e^2$  was used to include all the causes for half sisters resembling each other except that they are by the same parent.

of that word, and therefore where neither indicator is highly accurate! Values of  $h^2g^2$  less than .10 are not shown in either figure 2 or figure 3. For such values  $B^2$  would become very large if  $e^2r_{EE}$  were also very small.

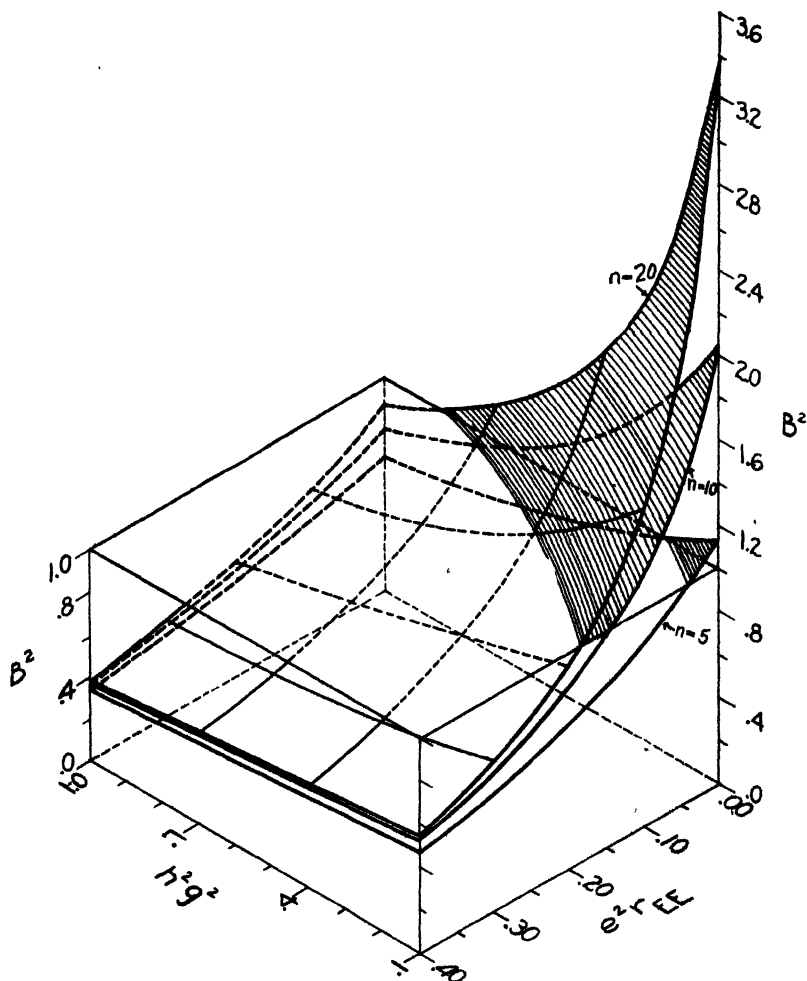


FIG. 3. The relative accuracy of progeny test and individual's own performance for three selected values of  $n$  (20, 10 and 5) for traits in which the importance of heredity varies and for populations in which the other reasons for offspring resembling each other vary from zero to a correlation of  $+.40$  between the offspring for other reasons than that they are out of the same dam.

Figure 3 shows how  $h^2g^2$  and  $e^2r_{EE}$  affect  $B^2$  for three selected values of  $n$  (5, 10, and 20). The shading shows the regions where  $B^2$  rises above 1.0.

The limiting effect of  $e^2r_{EE}$  on the accuracy of the progeny test is vividly shown in this figure. Only rarely can the accuracy of the progeny test in a whole population average as high as that of the parent's own record, if the correlation between herd mates due to herd environment is as high as .10 and if  $h^2g^2$  is as large as .10.

#### CONDITIONS WHICH MAY AFFECT THE VALIDITY OF THE SIMPLE SOLUTION

Figures 2 and 3 show the situation when  $m$  is one, each daughter is represented by one record only, breeding is at random, the sires are of unknown breeding value, dams and daughters are produced by the same kind of breeding system, and dams are just as representative of their generation as daughters are of theirs. Now these conditions will never be fulfilled exactly, not even in experimental data. It is important to glance for a moment at the way deviations from these conditions will affect figures 2 and 3, although only deviations from the last condition can alter those figures materially in the direction of making the progeny test more reliable.

When  $m$  is more than one—and it usually will be if  $n$  is as large as two—the dam's record will be more reliable, in accordance with the formula in equation (1). When each daughter is represented by an average of several records, the effect is that of increasing  $h$  and making the progeny test more reliable but this is usually more than offset by the increase in  $m$ . Any dam who has several daughters, each with more than one record, is almost certain to have had many records of her own, so that the average number of records per daughter will nearly always be less than  $m$ . Hence the use of an average of all available records will tend to make both indicators more reliable but will usually increase that of the dam more than that of the progeny.

Deviations from random mating may be of two kinds so far as they affect this problem. First there may be a correlation between the mates of a cow, irrespective of whether they are like or unlike her. A positive correlation of this kind has the same effect as an increase in  $e^2r_{EE}$  and therefore lowers the reliability of the progeny test. A negative correlation of this kind (*i.e.*, some degree of success in deliberately seeing to it that each cow was mated both to very good bulls and to an equal number of very poor bulls) would increase the accuracy of the progeny test slightly since it would eliminate some of the chance existing under truly random breeding, that one cow would happen to be mated only to good bulls, another only to poor bulls, etc. However such a negative correlation cannot be high if  $n$  is large, because if a cow is mated to three or more bulls those bulls cannot each one be exactly opposite to every other one! Moreover it is hard to imagine that such a general negative correlation could exist under any circumstances except a breeding experiment where it was made

a cardinal point to get from each cow daughters by poor bulls and daughters by good bulls.

The second kind of deviation from random mating would be a general correlation between dams and sires so that it would generally be true through the whole breed that good cows were usually mated to good bulls, poor cows to poor bulls, etc. This could come about either through inbreeding or through deliberate assortative mating based on individual performance or the performance of near relatives. It is not unreasonable, either from the genetic principles involved or from the published studies of dairy data to suppose that this correlation within dairy breeds might well be as large as  $+ .15$  or  $+ .20$ . So long as all breeders are working for high production, this correlation could hardly rise much above  $+ .25$  or  $+ .30$  without the practice of extreme inbreeding. The effect of such a general resemblance between sire and dam would be to increase the accuracy of the progeny test slightly, since each offspring would then tend to be like the dam, not only because of receiving half its inheritance from her but also because the other half of its inheritance came from a sire whose inheritance was more like that of the dam than if he had been chosen at random from all sires of the breed. A general correlation of  $+ .25$  between the genotypes of sires and dams would modify equation 2 enough that the progeny test might possibly be more accurate than the dam's own record while  $n$  is as small as 3, but this would be true only if  $e^2r_{EE}$  were zero and  $h^2g^2$  were very small, and there were no other correlation between sires. Since it is not often that all three of these conditions will be fulfilled, it seems unlikely that such deviations from random mating as exist in actual dairy cattle populations will often alter figures 2 and 3 enough to make the progeny test in a whole population average as accurate as the cow's own record when the cow has as few as four daughters. It should be mentioned that in plants where self-fertilization is possible, the resultant perfect correlation between genotypes of sire and dam is quite attainable and makes it possible for a progeny test based on as few as two offspring to be as accurate as the parent's own record. This is doubtless the most important fundamental reason that plant breeders make so much use of the progeny test, particularly when working with self-fertilized organisms like wheat.

Sometimes the merits of the different sires will be partly known. If their genotypes were perfectly known and there were no correlation between sires and dams, formula 2 would become:

$$B^2 = \frac{n}{4 + (n-2)h^2g^2 + 4(n-1)e^2r_{EE}} \quad (3)$$

Thus even such extreme knowledge as this would have but a trifling effect on figures 2 and 3. If mating is not at random, such information about the sires is quite useful in that it would cancel and prevent errors which would

otherwise be introduced by any such correlation between sires as that discussed in the next to the last paragraph. Knowledge of the sires therefore prevents a correlation between sires from causing the accuracy of the progeny test to fall far below the levels pictured in figures 2 and 3, but cannot of itself raise those levels much higher than is shown. If there is a general correlation between sires and dams and the merit of the sires is known from other evidence, then such knowledge will help to predict the dam's genotype but such help is because of that direct correlation between the genotypes of sire and dam, and not because of evidence from the progeny themselves.

Where dam and daughters have been produced by different breeding systems, the values of  $g$  for dam and daughter may not be identical and hence will not cancel out of equation 1 as has been assumed so far. The most extreme example of this, so far as concerns dominance, would be the case where the progeny test is conducted by breeding the cows to homozygous recessive bulls. In such a case the deceiving effects of dominance would become zero for the daughters and a correspondingly higher percentage of the total variance among them than among the dams would be truly genetic in the narrow sense of that word. Naturally such a test could not be made for a cow on account of her short life and limited reproductive-ness, but a corresponding test of a bull might be practical if the information gained were worth the cost of getting it. However dominance is usually only a minor cause for actual records deviating from breeding value. The assembling and maintaining of a herd of recessive cows for such tests would be expensive, even if possible at all. Moreover such a plan does not appear to offer any help for avoiding deception by epistatic effects. These considerations make it seem quite unlikely that such a testing plan would often yield information worth the cost of getting it. If the sire and dam belonged to different breeds, there would be increased probability of important misleading epistatic effects which would probably preclude using for testers individuals of a breed developed for other purposes, as for example the use of beef-bred cows for testing the breeding value of a dairy bull for milk and fat production.

Some of the most brilliant research in genetics, particularly in the genetics of *Drosophila* and of maize, owes its success to the devising and use of tester strains so designed as to eliminate (except for Mendelian sampling errors) the effects not only of dominance but also of epistatic interactions, thus making the truly genetic portion of the variance ( $h^2g^2$ ) among the offspring much higher than was the case among their parents. However the devising of such a tester strain has for its prerequisite a reasonably accurate hypothesis as to the number and kind of genes involved and (at least so far as concerns epistasis) of the precise way in which the effect of the gene combination differs from the sum of the average separate effects

of the genes concerned. The limited reproductive rate of farm animals, their comparatively long interval between generations and their expensiveness make it well nigh a lifetime's work to create such a tester strain, even if the investigator were wise enough or lucky enough to have chosen the correct working hypothesis at his first attempt and even if the genetic situation were simple. Hence it appears unlikely that much use can be made of such tester strains in the breeding of farm animals, however useful and even necessary such tester strains may be in research on fundamental principles of genetics.

The assumption that daughter and dam are equally representative of their generation and that their records were selected by methods equally fair to both will often be untrue especially in semi-commercial data, and is the only one of these assumptions likely ever to be important enough in a whole population to make much change in figures 2 and 3. The effect of such differences between daughters and dams is to make  $h$  in figure 1 unequal to  $h_p$ . If they are unequal these two terms cannot be cancelled out of equation 1 as has been inferred hitherto.

If but one record is used to represent each animal and if that record is selected on some such *ex post facto* basis as the choice of the highest record after having seen them all, such a selection deliberately picks out that record made under the most favorable combination of unknown conditions of environment and health. Such selection automatically tends to give to the cow having many lactations a figure less truly representative of her phenotypic producing ability than would be the case with a cow which had but one or two lactations from among which to select. Since the dam usually has more records than the daughter, the net result is to make the dam's record usually less typical of her than the daughter's record is typical of her. This tends to make the progeny test more accurate than is pictured in figures 2 and 3. It should be pointed out that this comes not from any intrinsic superiority of the progeny test, but rather from the bias introduced by the choice of records where variable numbers are available. Studies of the actual correlations between records of the same cow in different lactations in the same herd have generally given values ranging from about +.30 to +.60. Correlations as low as this give ample room for selections of the largest record for each cow to cause such selected records to deviate far from each cow's phenotype, especially if some cows have only one or two records and others have 4 or 5, from among which to select.

Probably more generally important is the fact that the dams are often a more highly selected group than the daughters are. Usually all of the progeny which were tested at all are included in the progeny test. If they were very poor producers, they may have been culled shortly afterward but their records remain for use in the progeny test. The conditions may be quite otherwise among the dams. Not all cows which are tested succeed

themselves in becoming dams of tested daughters. The dams being studied in any particular set of data are to some extent only the more favored survivors from among the group tested contemporarily with them. In studying correlations between the records of those dams and any measure of the subsequent performance of their progeny, one is by no means making a comparison between phenotypic and genotypic manifestations of production. Instead he is comparing the utility of those phenotypic differences still remaining among the dams after this selection has been practiced, with the utility of various averages of phenotypic differences among the less highly selected group of daughters. This seems to be a major part of the explanation for the practical findings of Jull (6, 7) and Copeland (1) that the ancestor's production record is of relatively limited value in breeding selections. In Jull's data all birds which laid less than 200 eggs were culled before they had a chance to become dams. What would have happened, if all hens or a strictly random sample of those hatched in each generation had been progeny-tested, of course can only be surmised. Copeland's data came from many herds and at various dates, so that selection was not by such uniform rules as in Jull's experimental data. Nevertheless Copeland's tables 1 and 5 show that there had been stronger selection among the dams than among either their daughters or granddaughters, else the extreme regression (about 50 to 70 pounds of fat) from the dams' average to the daughters' and granddaughters' averages would not have occurred.

Naturally such studies as those of Jull and Copeland (many others might be mentioned but these two are selected as typical of the more careful among recent ones) correspond closely to the conditions practical breeders actually face. Depending on the amount of selection involved and on how highly hereditary the trait really is, it may sometimes be true that such variation as remains among a highly selected group of dams may be less useful as an indication of the hereditary differences between those dams than the variation which exists among the relatively unselected daughters of those dams, even when there are as few as two daughters per dam. The point to be made here is that when this actually is the case, it is not because of any inherent biological superiority of the progeny test but rather it is because in the case of the dams we are comparing what *further selection* could do among a group of survivors on which considerable selection has already been practiced, with all which selection could do among a group of daughters where very little selection has yet been practiced. The practical utility of such comparisons is beyond argument for the breeder who must actually use, in some combination or other, some data from the more heavily selected population and some data from the less selected population. There is, however, some danger of confusion as to *the utility of the initial selections* in the populations from which the dams first come, if such studies are interpreted as meaning that an individual's own record is in-

herently less representative of its breeding value than the average records of as few as two or three of its offspring.

#### GENERAL CONSIDERATIONS AND DISCUSSION

The general conclusion to be drawn from all these considerations is that only under rare and unlikely combinations of conditions would a progeny test based on as few as four-daughters-average in an unselected population as accurate an indicator of a dam's breeding value as the dam's own performance. If the trait is highly hereditary, in the narrow sense of that word, or if the progeny resemble each other very much for reasons of having been exposed to a common environment, many more than four daughters may be required or it may even be quite impossible for the progeny test to average more accurate in a whole population than the dam's own performance, no matter how many daughters there are.

The superiority of the progeny test is greatest for traits which are least hereditary in the narrow sense of that word. For traits which are very faintly hereditary and for which the offspring do not resemble each other for reasons of having been under common environment, the progeny test can become several times as accurate as the dam's own record, but for that to be true the trait must be so slightly hereditary that neither indicator can be highly accurate!

The progeny test is needed most where one sex cannot express the trait, as in milk and fat production in dairy cattle, egg production in poultry, prolificacy in swine, etc. Since the biometrical relations between sire's genotype and progeny average are the same as those between dam's genotype and progeny average (except for sex-linked inheritance which must be a small part of the total inheritance in mammals) the foregoing considerations apply also to the progeny test of the sire. However, for traits which the sire cannot express himself, there is nothing but a pedigree estimate of the sire against which to compare the accuracy of a progeny test of him. Since a progeny test surpasses even the best pedigree estimate when there are more than three progeny (provided the offspring do not resemble each other very much for any other reason except that they are by the same sire), the progeny test in such cases is much more useful and more urgently needed for the sire than for the dam. However such progeny tests will rarely tell as much about the sire as the available information correctly used will tell about a dam of nearly equal age. Enthusiasm over some such slogan as the current one that "The next best thing to a proved sire is the son of a proved sire" should not cause us to forget that such a son is one generation of Mendelian segregation away from this sire and that half of his inheritance (a little more than half if the probably small amount of sex-linked inheritance in mammals is also considered) comes from his dam

whose breeding value may usually be estimated more closely than that of his proved sire, if the available information is fully and fairly used.

Although the progeny test will not often be *superior* to the dam's own record (where no differences of selection are involved), it will be noticed from equation 2 that  $B = .5$  even when  $n$  and  $m$  are only one. Therefore even the most fragmentary progeny test is worth much as an indicator of the dam's genotype. Naturally, as in any other prediction, both indicators should be used where both are available. The principles of multiple correlation govern the amount of attention to be paid to each indicator where both are used. However the formulae for the relative amount of attention to be given to the two records are not only complex but also involve a term (which is usually indeterminable) for the degree to which the dam's record and her daughters' records resemble each other because of being made under common environment. The ratio between the standard regression coefficients will be in the same direction from unity as is the ratio between the primary correlations ( $B$  in the earlier algebraic discussion) but will be more extreme. Perhaps as good an approximate rule as any would be to give a daughter's production nearly half as much attention as the cow's own record where there is only one record for each, but to give between  $\frac{3}{8}$  and  $\frac{1}{2}$  as much attention to the daughter average as to the cow's own record where there are at least two or three offspring. This rule would be approximately correct if daughters and dams had been equally selected from their generations. Where the dams have already been highly selected, more importance than this should be given to the progeny test in making further selections but it does not seem possible to develop any simple general rule for this, since such a rule would include terms for the intensity of selection among dams and among daughters and also a term for the extent to which the trait is really hereditary.

In actual practice selection will also be based in part on pedigree. Chronologically pedigree becomes available first, then the individual's own performance and the progeny test comes latest. As some selection is practiced on each basis, the possibilities of further gains by additional selections on the same basis rapidly diminish unless new information becomes available. The sampling nature of Mendelian inheritance sets a lower limit on the usefulness of pedigree than is inherently necessary for either of the other two bases of selection. Hence the very early selections on the basis of pedigree come near exhausting the possibilities in that direction, although increased knowledge of the performance of parents and other ancestors or collateral relatives does make it profitable occasionally to revise an earlier pedigree estimate. On the other hand, knowledge of the individual's own performance can continue to increase at a practically important rate as long as it lives and knowledge of its progeny can increase

as long as previously untested progeny come on test and (at a slower rate) as long as progeny already tested continue to be tested at later ages.

This leads to the general picture that pedigree occupies first place only for such selections as must be made before the other two criteria are available. It is very distinctly in third place after a few such early selections have been made. Individual performance occupies first place after it becomes available and until considerable use has been made of it. Then progeny test is the most useful basis for further selections but when it is much used for such selections, the possibilities in that tend to be exhausted and the individual's own performance might again assume first place. Among mature animals under practical conditions individual performance and progeny test therefore will vary or alternate as most useful for further selections, according to the amount of new knowledge becoming available about each and according to how nearly the possibilities for selection on the basis of the existing knowledge about each have already been exhausted.

#### SUMMARY

1. The biometrical relations governing the relative accuracy of progeny test and of the parent's own performance as indicators of the parent's breeding value are presented and discussed.

2. A solution under the simplest conditions is presented algebraically and graphically. Under those conditions there must be at least five offspring before the progeny test in a whole population will usually be a more accurate indicator of the parent's breeding value than the parent's own performance.

3. Most deviations from those simplest conditions have only slight effects on the solution. However any general resemblance between the offspring for any other reason than that they are half-sibs through the parent in question sets serious limits on the accuracy of the progeny test. On the other hand if the parents or the records used to represent them are more highly selected than the offspring or their records, the progeny test may become relatively more accurate than under the simple conditions for which the algebraic solution is given.

4. The progeny test is needed most for traits which cannot be expressed in one sex and for traits which are but slightly hereditary.

5. The bases for estimating breeding value are pedigree, own performance, and progeny test. As fast as some selection is practiced on one of these bases, the possibilities for further progress by additional selection on the same basis rapidly diminish and correspondingly increased attention should be given to one of the other bases.

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# ELECTROKINETICS IN RELATION TO DAIRY PHENOMENA.

## 1. THEORY AND METHOD

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For a long time it has been known that a number of dairy phenomena depend upon the coalescence or non-coalescence of the fat globules. Such dairy problems as the creaming of milk, the viscosity of cream and tendency to form cream plugs, the tendency of bottled cream to show a skim milk layer and the whipping ability of ice cream mixes are at present definitely known to involve the clustering of fat globules.

In the past a considerable amount of experimental work has been done on the practical factors that influence the above mentioned phenomena. Such studies, while of great value and importance, do not reveal the fundamental factor or factors that are operative.

Within recent years, it has been suggested by several investigators that the electrical effects at the fat-skim milk interface may be the governing factor, or at least one of the important factors in the behavior of milk fat globules under various conditions. With this suggestion in mind the present investigation was carried on.

### 1. HISTORICAL

Up to the present time, the literature concerning the electrical potential at the surface of the fat globule of cow's milk is very meager. Various references refer to such a charge, but most citations have been made purely on a speculative basis. However, a few references based on experimental work are available in the literature.

Sirks (28) in 1924 made a few observations on the electric charge of the fat globules. He concluded, from his preliminary work that the charge on the fat globule was of a low order of magnitude, and he was unable to correlate the charge with any dairy phenomena.

Mommsen (22) in 1929, using cataphoretic means, investigated the charge on the surface of the fat globule of cow's and woman's milk. He concluded that the fat globules of milk are normally negatively charged, in that they migrate in the direction of the anode when placed in an electrical field. Two experimental methods were used: first, the migration in "U" tubes was noted, and second, the pH was determined at which the iso-electric point was established in buffer mixtures.

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He found that the normal iso-electric point of the fat globule for cream diluted 1:100 was established at a pH of 4.17. While calcium ions added in the form of calcium chloride caused the iso-electric point to be raised. The addition of Ringer's solution to the buffer mixture caused the iso-electric point to be shifted to the acid side of pH 4.17. Additions of gelatin caused a shift to the iso-electric point of gelatin. This shift, he points out, was more evident with increasing concentrations of gelatin. The iso-electric point of the fat globules of human milk was found to be on the acid side of pH 4.17. This agrees with the recent work of Nugent (23) who found the iso-electric point of pH 4.1.

Prieger (27) has used cataphoretic means to study the nature of the hull surrounding the fat globule; her results, as in the case of Mommsen, are expressed in terms of the pH of the iso-electric point. She points out that the iso-electric point of artificial protein-free fat emulsions is more on the acid than is the case with the natural fat globules in milk. Because of this difference of hydrogen-ion concentration necessary to establish the iso-electric point she concludes that the natural fat globules of milk are surrounded by protein-like films, while the protein free artificial fat emulsion displays the true charge of the fat globule.

That the fat globules are surrounded by a layer of protein material, is in agreement with the findings of Titus, Sommer, and Hart (31) who arrived at the above conclusions by determining the nitrogen distribution of the fat "hulls" that were obtained by washing the fat globules. Hattori (15) (16) is of the opinion that the composition of the fat "hulls" is different from any of the other known proteins. Palmer and Wiese (32) have also investigated the "hull" material and conclude that it is composed of a mixture of protein and phospholipides.

Mohr and Brochmann (21), also using cataphoretic methods, have recently investigated the charge on the fat globule. These workers found a relationship between the churning ability of the cream and the charge on the fat globules. They did not, however, find any appreciable effect of such treatments as heating or homogenization on the pH of the iso-electric point. Neither did the addition of emulsifiers have any effect. They found that the iso-electric point of pure butter fat emulsions was more on the acid side than that of the fat emulsion in normal milk.

## II. THEORY

The fact that colloidal systems, when placed in an electrical field, show the phenomenon known as cataphoresis was first firmly established by Linder and Pieton (19) in 1892. Hardy in 1900 (13) noted that a suspension of egg albumin was positively charged (migrated to the cathode) in acid solution; but when placed in an alkaline solution it was negatively charged. In both cases the suspensions were stable, but when no migration

took place flocculation resulted. The same phenomenon was later observed by Burton (2).

As a result of cataphoresis experiments, Hardy (13) expressed the opinion that the presence of an electrical charge on the surface of a particle was an important factor governing its stability, since like charges on the particles would tend to keep the particles apart because of their mutual repulsion, and at the iso-electric point flocculation would occur. Powis (26) later pointed out that it was not necessary to bring the charge down to zero but only to a certain low order of magnitude (which he calls the critical potential) in order to bring about flocculation.

From such investigations as these, it has become quite generally accepted that the electrical charge is an important factor in the stability of colloidal systems.

However, it should be pointed out that in the case of one type of colloids known as emulsoids, there is an additional factor that must be considered. This factor is called by some the hydration of the particle. In order for flocculation of such a system to take place, it is necessary that both the electrical charge and the hydration factors be overcome or removed. In the case of emulsions a third factor, that of the emulsifying agent, must also be considered.

The original conception of Helmholtz (17) that the electrical charges existed in the form of a double layer, has been modified by Gouy (9) in that the charges constitute an ionic atmosphere. Such an "atmosphere" is not fixed but movable; the charged particle is free to move under the influence of an applied electromotive force. In cataphoresis we are concerned with the movement of particles suspended in a liquid under the influence of an applied potential. Streaming potentials refer to the E.M.F. set up by the movement of a liquid past a wall. Both types of electrokinetic phenomena are mathematically related.

Perrin (25), Briggs and Gortner (1) have derived the following expression of the streaming potential:

$$\xi = \frac{HK_s 4\pi N}{PD}$$

$\xi$  = zeta potential  
 $H$  = observed E.M.F.  
 $K_s$  = specific conductivity  
 $N$  = viscosity  
 $P$  = applied pressure  
 $D$  = dielectric constant

It has recently been argued by Delbye and Hückel (3) that for a spherical particle the factor in the above equation should be six instead of four.

Krout (18) and Freundlich (7) have reported results of experimental work on the streaming of liquids through capillary glass tubes. The liquid was forced through under a measured hydrostatic pressure and the electro-

motive force that was set up was measured by suitable means. Their findings show that the observed electromotive force was independent of the cross section area or length of the tube. For a given liquid the E.M.F. that was set up was proportional to the applied pressure. However, they found that this value varied with the kind of glass that was used, but that the observed potential was constant for all shapes and sizes of tubes that were prepared from the same kind of glass.

Later Gee and Harrison (8) and Harrison (14) used the streaming potential method in developing their electrical theory of dyeing. They determined the potential of silk, wool and cotton against solutions of various electrolytes and dyestuffs. Fibers were packed in a cell between platinum electrodes and the E.M.F. that was set up when the liquid was forced through this diaphragm was measured.

Their findings show that acids increased the charge of positively charged fibers and decreased it on negatively charged fiber. All acids had the same effect, when the hydrogen-ion concentration was the same. Bases acted exactly opposite to that of acids. When an electrolyte containing polyvalent ions of opposite sign to that of the fiber was added, the charge was lowered, while in some cases it was even possible to reverse the sign.

Recently Briggs and Gortner (1) have used the streaming potential method for determining the potential existing at the cellulose-water interface. Gortner and his students (11) (12) have used the method extensively in studying the properties of the interface, including molecular configuration. Their method consists of forcing water, under a measured hydrostatic pressure, through a diaphragm of cellulose that is held in place by two perforated gold electrodes. In order to overcome the effects of polarization a quadrant electrometer was used.

It was pointed out, that for accurate measurements it is necessary to measure the specific conductivity of the liquid while it is in the diaphragm because "surface conductance" is quite an important factor in such electrokinetic studies.

### III. DEVELOPMENT OF METHOD

Preliminary experimental work was carried on by a number of cataphoretic methods. However, these methods failed to give satisfactory results and were therefore discarded.

After a consideration of various other methods, it was decided to use, if possible, a cell similar to that used by Briggs and Gortner (1) in their studies on the electrokinetic potential at the cellulose-water interface. In their method, a liquid was forced through a diaphragm of cellulose that was held in place by two perforated electrodes. It was thought that such a cell could be constructed in which cream could be placed (being kept within the electrodes by a fine grade of filter paper) and skim milk could be forced

through, and any potential that might be established could be measured by suitable means. This method likewise proved very unsatisfactory. After a short time the filter paper would become clogged, thus preventing any streaming of the liquid.

Upon a suggestion of Gortner (10), it was thought possible to force the skim milk through a small hole in an electrode that was sealed in the end of a glass tube. A fine stream of skim milk could therefore be made to impinge upon another electrode that would serve as a target. The space in the immediate vicinity could then be filled with melted butter oil. Such a method, would in reality be the same as forcing skim milk through a fine "butter oil" capillary. Such a cell was constructed and a considerable amount of work carried on with it. However, it had the disadvantage of only being able to make measurements at comparative high temperatures, since it was essential that the butter oil be kept in the liquid state. Further, such an apparatus did not allow for complete adsorption of the milk colloids and salts at the interface and therefore the conditions were not identical with that of the normal fat globules as they exist in milk. Some difficulty also was experienced in keeping the pressure uniform.

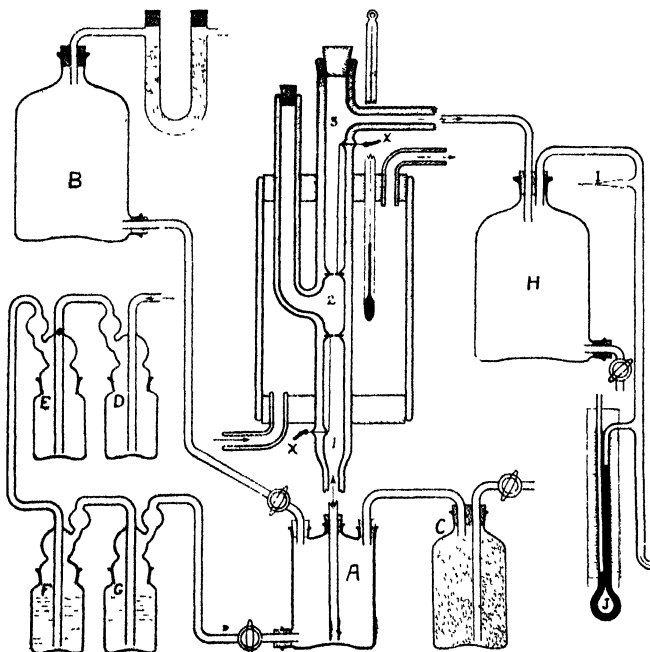


FIGURE 1

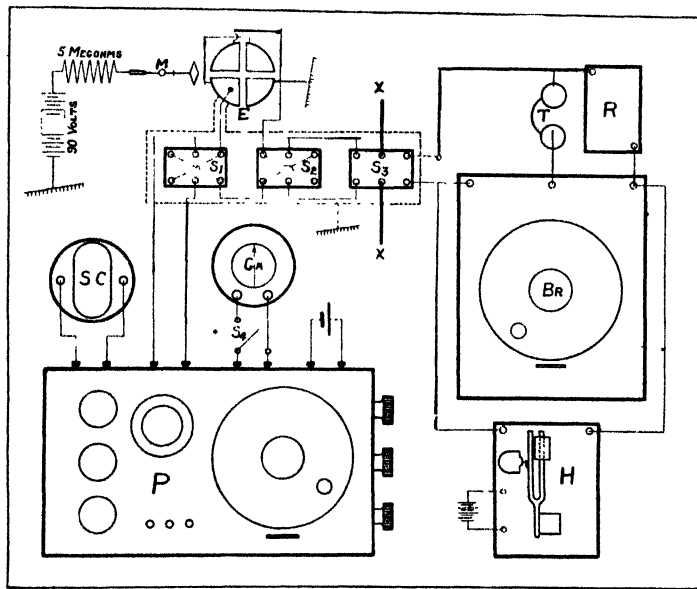
To facilitate adsorption at the fat-liquid interface a special streaming potential cell was developed. A sketch of the apparatus is shown in figure 1. The capillary was formed by placing a fine, long needle through two platinum electrodes that were sealed in a heavy glass tube. The chamber (2) was then filled with melted butter oil which was then chilled to solidify it. By carefully removing the needle a butter oil capillary was thus formed. The capillary and chambers 1 and 3 were then filled with skim milk to allow for adsorption of the milk colloids to take place. After fifteen minutes the excess skim milk was removed by passing a stream of conductivity water through the cell. The preparation of the cell and the actual streaming potential measurements were made at a constant temperature by circulating water from a bath through the outer jacket.

In solutions which possess considerable conductivity the charge is partly neutralized; diluted solutions were therefore used. The sample which was to be subjected to streaming potential measurement was prepared by placing 10 cc. of a 1 to 1,000 dilution of skim milk in conductivity water, in Wolff bottle (A). To this, 100 cc. of conductivity water were added from reservoir (B). Air was now passed through wash bottles containing sulphuric acid (D), sodium hydroxide (E and F) and water (G), and admitted into bottle (A). This washed air was bubbled through the sample fifteen minutes to mix it, to remove any dissolved carbon dioxide, and to replace the air in the bottle. A controlled vacuum was then applied to draw the sample through the butter oil capillary. After passing through the capillary the liquid was collected in bottle (H). While liquid was being withdrawn from bottle A, air was admitted through a soda lime trap (C) rather than through the wash bottles so that the hydrostatic pressure could be accurately measured. The vacuum was regulated by the valve (I) and measured by manometer (J). Readings were taken at several different pressures to check the accuracy.

A sketch of the electrical apparatus used for measuring the potential and conductivity is shown in figure 2. The electromotive force (electrical leads marked X) that was set up at the ends of the capillary by the streaming liquid was measured by a type K-2 Leeds & Northrup potentiometer (P) and a carefully shielded Compton electrometer (E). In this procedure the potentiometer was first adjusted by balancing the standard cell (S.C.) against a two volt storage battery by means of the rheostat provided for that purpose. After this the galvanometer (G) was shorted out by means of switch (S4). Any desired voltage could then be taken from the potentiometer and opposed to the potential to be measured. With the electrometer (E) in the circuit of the unknown potential, the potentiometer voltage was varied until the electrometer indicated that the unknown potential had been exactly balanced. The potentiometer reading thus indicated the unknown potential. By using the electrometer as the null point instru-

ment polarization effects are eliminated or reduced to insignificance. The switches ( $S_1$  and  $S_2$ ) were provided for reversing the quadrants of the electrometer, and the poles of the cell respectively.

The conductivity measurements were made by means of the conventional Wheatstone-bridge method. To permit rapid switching from potential measurements to conductivity measurements the electrical leads X from the cell were connected to the switch ( $S_3$ ). The high frequency current of one thousand cycles was produced by the microphone hummer (H); (Br) was a drum type Wheatstone bridge, (R) variable resistance and (T) the tunable telephones.



## FIGURE 2

It will be noted that the results are not expressed in terms of the final zeta potential but as values of  $Hk_s/P$  (the observed electromotive force times the specific conductivity divided by the applied hydrostatic pressure). This expression was used since the remainder of the equation for calculating the zeta potential should be practically constant when such dilute solutions are used. However, it must be conceded, that in measurements made at lower temperatures, a slight error arises due to increased viscosity. Furthermore, since the values of the other terms of the equation for the solutions used were not accurately known, it was deemed advisable to express the results in terms of the above three mentioned variables.

Where hydrogen-ion concentrations were to be measured a Clark electrode vessel was used. To exclude carbon dioxide of the air, the vessel was

filled in the following manner: The funnel was closed with a two-holed rubber stopper which provided a vacuum connection and a connection to bottle (A). Hydrogen was passed through the vessel, through the funnel and the rubber tubing to replace all air. Suction was then applied to draw a suitable portion of sample into the funnel. By proper manipulation of the stop-cocks this sample was allowed to flow into the vessel and the excess hydrogen was allowed to escape at the opposite end. With the hydrogen in the vessel at atmospheric pressure, the vessel was shaken in the usual manner to establish equilibrium.

With highly dilute and poorly buffered solutions the usual potentiometric measurement leads to inaccurate results on account of the polarization involved in attaining the point of balance. This was avoided by using the same procedure for measuring this potential as has already been described for measuring the streaming potential.

#### IV. EXPERIMENTAL

The butter oil that was used in all of the experiments was prepared from unsalted, sweet cream butter taken directly from the churn. It was melted at approximately 45° C. to 50° C., care being taken never to heat it beyond the above mentioned temperatures. The curd was then allowed to settle to the bottom of the container and the oil was filtered twice through a standard grade of filter paper.

The skim milk was taken from the power separator in the University Creamery. All of the skim milk was diluted with one thousand parts of conductivity water.

Table 1 and figure 3 show the relationship between the applied hydrostatic pressure and the electromotive force that was set up. Theoretically these points should fall in a straight line, and as can be noted the points tend to form (within experimental error) a straight line.

In an effort to determine whether the charge was that of the fat plus the adsorbed proteins or just the fat, an experiment was run first measuring the potential at the fat-liquid interface. The capillary was then allowed

TABLE 1  
*Showing relation between pressure and electromotive force*

PRESSURE	E.M.F.	H/P
<i>mm.</i>	<i>millivolts</i>	(-)
111	480	4.3
147	650	4.4
180	800	4.4
220	950	4.3
294	1280	4.3
333	1400	4.3
418	1800	4.3

to stand in contact with skim milk for a period of fifteen minutes, the unadsorbed material washed away, and the potential determined. The results are shown in table 2.

It was desired to determine the electrokinetic potential of samples of individual skim milk. The results are shown in table 3. As will be noted, the potential is negative and there is some variation even in mixed samples.

In order to make a comparison of the iso-electric points with those reported in the literature, that were determined by other methods, the

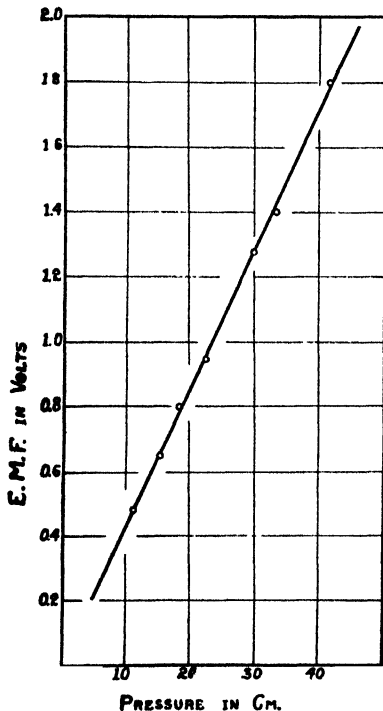


FIGURE 3

hydrogen-ion concentration at the point of zero potential was determined. The results are shown in table 4 and figure 4. It will be noted that the iso-electric point was found to be at approximately pH 4.3.

Tables 5 to 10 inclusive, and figures 5 and 6, show the effect of various salts on the magnitude of the electrical potential. It will be noted that the first four salts are all chlorides and that the valence of the other ion increases from one to four. The last two salts used contain the citrate and phosphate radical, respectively. The chloride salts tended to reduce the charge depending on the valence of the other ion. On the other hand, citrates and phosphates increased the already negative charge.

TABLE 2  
Potentials at the fat-liquid interface compared with the fat-milk colloid-liquid interface  
SERIES NO. 1

	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
	<i>mm.</i>	<i>millivolts</i>		<i>Mhos</i>	(-)
Fat-liquid interface	147	600	4.0	1.36	6.04
	180	750	4.1		
	220	900	4.0		
Fat-milk colloid-liquid interface	147	380	2.5	1.90	4.75
	180	450	2.5		
	220	600	2.5		

SERIES NO. 2

	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
	<i>mm.</i>	<i>millivolts</i>		<i>Mhos</i>	(-)
Fat-liquid interface	147	990	6.0	1.41	8.60
	180	1100	6.1		
	220	1400	6.3		
Fat-milk colloid-liquid interface	147	600	4.0	2.01	7.84
	180	700	3.8		
	220	850	3.8		

TABLE 3  
Electrokinetic potentials of various skim milks from individual cows

SAMPLE	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
	<i>mm.</i>	<i>millivolts</i>		<i>Mhos</i>	(-)
No. 1	147	650	4.4	1.89	8.13
	180	800	4.4		
	220	900	4.0		
No. 2	147	365	2.4	2.11	5.48
	180	470	2.6		
	220	600	2.7		
No. 3	147	475	3.2	1.95	6.04
	180	600	3.3		
	220	660	3.0		
No. 4	147	600	4.0	1.90	7.41
	180	720	4.0		
	220	850	3.8		
No. 5	147	450	3.0	2.03	6.29
	180	570	3.1		
	220	690	3.1		
No. 6	147	510	3.4	1.96	6.84
	180	630	3.5		
	220	750	3.4		
No. 7	147	600	4.0	1.91	7.45
	180	700	3.8		
	220	875	3.9		
No. 8	147	390	2.6	2.10	5.67
	180	490	2.7		
	220	600	2.7		

Table 11 and figure 7 show the effect of temperature on the potential. It will be noted that almost a straight line function exists between the electrokinetic potential and the temperature of the measurement.

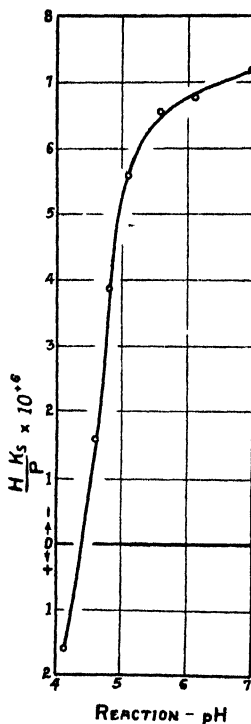


FIGURE 4

#### V. DISCUSSION

The experimental data reported here show that the potential at the fat-liquid interface is negative, and that it is slightly less negative after opportunity and time have been provided for the adsorption of milk colloids at the interface (Table 2). These results are in harmony with the findings of previous workers; the negative potential or charge on fat globules has been reported by several investigators (22, 27, 21); the effect of adsorbed milk colloids is indicated by the work of Prieger (27). Prieger determined the iso-electric point of fat globules in fat-water and fat-skim milk emulsions. She found that a lower pH was required to attain the iso-electric point in the fat-water emulsions than in the fat-skim milk emulsions.

It has been known for some time through cataphoresis experiments that a negative charge exists at the interface when oil drops are suspended in pure water. Since pure water contains only hydrogen and hydroxyl ions,

TABLE 4

pH	PRESSURE	E.M.F.	H/P	$\kappa_a \times 10^{-4}$	$HK_a/P \times 10^{+4}$
	<i>mm.</i>	<i>millivolts</i>		<i>Mhos</i>	
	147	675	4.5	1.56	7.02 (-)
	180	800	4.4		
	220	1000	4.5		
6.14	147	590	4.0	1.71	6.84 (-)
	180	725	4.0		
	220	875	3.9		
5.63	147	490	3.3	2.01	6.63 (-)
	180	600	3.3		
	220	725	3.2		
5.10	147	300	2.0	2.83	5.66 (-)
	180	350	1.9		
	220	350	2.0		
4.81	147	200	1.3	3.05	3.96 (-)
	180	235	1.3		
	220	310	1.4		
4.68	147	75	0.5	3.31	1.65 (-)
	180	100	0.5		
	220	120	0.5		
4.12	147	60	0.4	4.02	1.60 (+)
	180	75	0.4		
	220	90	0.4		

TABLE 5  
*Effect of potassium chloride*

CONC.	PRESSURE	E.M.F.	H/P	$\kappa_a \times 10^{-4}$	$HK_a/P \times 10^{+4}$
$N \times 10^4$	<i>mm.</i>	<i>millivolts</i>		<i>Mhos</i>	(-)
	147	445	3.0	1.20	3.60
0.0	180	520	2.8		
	220	650	2.9		
	147	470	3.2	1.25	4.00
0.5	180	560	3.1		
	220	700	3.1		
	147	500	3.4	1.31	4.32
1.0	180	600	3.3		
	220	725	3.3		
	147	450	3.0	1.37	5.11
2.0	180	550	3.0		
	220	685	3.1		
	147	400	2.7	1.96	5.29
4.0	180	490	2.7		
	220	575	2.6		
	147	300	2.0	2.1	4.2
6.0	180	360	2.0		
	220	450	2.0		

TABLE 6  
Effect of calcium chloride

CONC.	PRESSURE	E.M.F.	H/P	$\kappa_a \times 10^{-6}$	$HK_a/P \times 10^{-6}$
$N \times 10^4$	mm.	millivolts		Mhos	(-)
0.0	147	450	3.2	1.11	3.50
	180	575	3.2		
	220	700	3.1		
0.5	147	385	2.6	1.23	3.07
	180	450	2.5		
	220	550	2.5		
1.0	147	290	1.9	1.29	2.45
	180	330	1.8		
	220	430	1.9		
2.0	147	75	0.5	2.32	1.39
	180	110	0.6		
	220	140	0.6		
4.0	147	60	0.4	2.98	1.19
	180	80	0.4		
	220	90	0.4		
6.0	147	30	0.2	3.37	1.01
	180	70	0.3		
	220	80	0.3		

TABLE 7  
Effect of ferric chloride

CONC.	PRESSURE	E.M.F.	H/P	$\kappa_a \times 10^{-6}$	$HK_a/P \times 1$
$N \times 10^4$	mm.	millivolts		Mhos	(-)
0.0	147	500	3.4	0.96	3.36
	180	630	3.5		
	220	775	3.5		
0.5	147	400	2.7	1.05	2.83
	180	480	2.6		
	220	600	2.7		
1.0	147	300	2.0	1.18	2.36
	180	370	2.0		
	220	450	2.0		
2.0	147	250	1.7	1.22	2.07
	180	300	1.6		
	220	375	1.7		
4.0	147	220	1.5	1.28	1.92
	180	275	1.5		
	220	350	1.6		
6.0	147	170	1.1	1.31	1.44
	180	200	1.1		
	220	250	1.1		

TABLE 8  
Effect of thorium chloride

CONC.	PRESSURE	E.M.F.	H/P	$K_a \times 10^{-8}$	$HK_a/P \times 10^{-8}$
$N \times 10^4$	mm.	millivolts		Mhos	
0.0	147	600	4.0	0.98	4.02 (-)
	180	750	4.1		
	220	950	4.3		
0.5	147	500	3.4	1.07	3.85 (-)
	180	700	3.8		
	220	800	3.6		
2.0	147	330	2.2	1.21	2.66 (-)
	180	400	2.2		
	220	500	2.2		
4.0	147	200	1.3	1.28	1.66 (-)
	180	265	1.4		
	220	300	1.3		
6.0	147			1.32	0.13 (+)
	180	10	0.05		
	220	22	0.10		

TABLE 9  
Effect of di-sodium phosphate

CONC.	PRESSURE	E.M.F.	H/P	$K_a \times 10^{-8}$	$HK_a/P \times 10^{-8}$
$N \times 10^4$	mm.	millivolts		Mhos	(-)
0.0	147	525	3.5	1.01	3.54
	180	625	3.4		
	220	775	3.5		
0.5	147	650	4.4	1.14	4.90
	180	750	4.1		
	220	950	4.3		
1.0	147	740	5.1	1.22	6.10
	180	900	5.0		
	220	1100	5.0		
2.0	147	620	6.2	1.56	9.51
	180	1100	6.1		
	220	1350	6.1		
4.0	147	1030	7.0	2.08	14.21
	180	1280	7.1		
	220	1550	7.0		
6.0	147	810	5.5	2.27	12.48
	180	1000	5.5		
	220	1200	5.4		

the negative potential is attributed to more extensive adsorption of hydroxyl ions than of hydrogen ions. In a fat-skim milk emulsion we have, in addition, the various ions of the milk salts, and the milk colloids. The potential that obtains at the interface must be the net result of the adsorption of the various ions, the adsorption of each ion reaching equilibrium at a definite concentration of that ion in the serum proper (reversible adsorption).

TABLE 10  
*Effect of sodium citrate*

CONC.	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{-6}$
$N \times 10^4$	mm.	millivolts		Mhos	(-)
0.0	147	650	4.4	1.07	4.70
	180	800	4.4		
	220	990	4.5		
0.5	147	750	5.1	1.19	5.72
	180	970	5.3		
	220	1160	5.2		
1.0	147	900	6.1	1.80	11.34
	180	1175	6.5		
	220	1400	6.3		
2.0	147	1000	6.8	2.21	15.25
	180	1300	7.2		
	220	1500	6.8		
3.0	147	1120	7.6	2.33	17.71
	180	1400	7.7		
	220	1650	7.5		
5.0	147	900	5.9	2.87	17.22
	180	1110	6.1		
	111	675	6.0		
7.0	147	750	5.1	3.01	15.05
	180	900	5.0		
	220	1025	4.9		
10.0	147	300	2.0	4.63	9.26
	180	360	2.0		
	220				

However, it must be expected that the conditions will be further complicated by the fact that some of the milk colloids are irreversibly adsorbed, thereby conferring new properties upon the interface. With a film of irreversibly adsorbed milk colloids on the fat globules ("fat hulls") we now have an interface in which the adsorbed milk colloids will probably play the dominant rôle in determining the potential.

That fat globules in milk possess "fat hulls" in the sense that there is irreversibly adsorbed material present has been demonstrated by the work of various investigators. In such work the method of isolating the "fat hull" material has in all cases involved the repeated washing of the globules with water, followed by the removal of the fat, leaving the "fat hull" material for study. In washing the fat globules, two methods have been used; in one method the fat globules, introduced as whole milk into the bottom of a tall column of water, are washed as they rise through the water; in the second method milk is separated centrifugally, the cream is suspended in

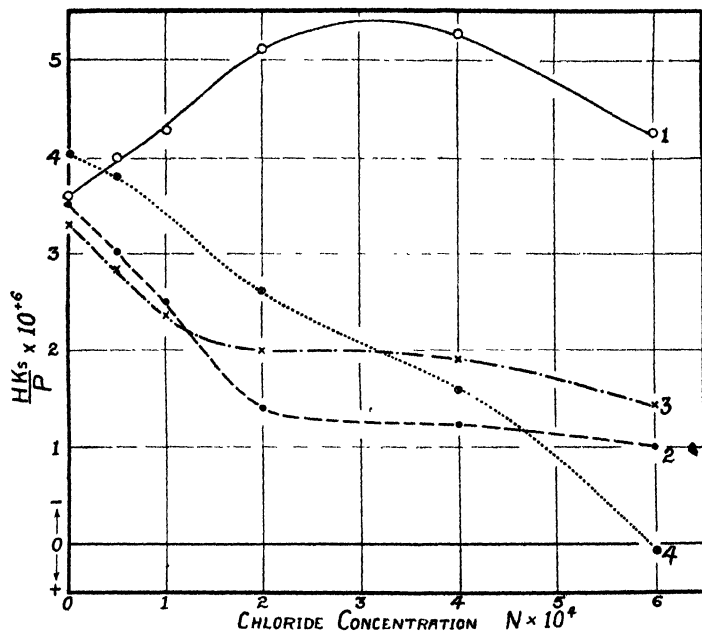


FIGURE 5

1 = KCl

2 =  $CaCl_2$ 3 =  $FeCl_3$ 4 =  $ThCl_4$ 

water and re-separated a number of times. In either method the fat globules are suspended in pure water for a sufficient length of time that reversibly adsorbed material must inevitably be removed. The study of "fat hulls" in the past has therefore included only material that is irreversibly adsorbed.

The chemical nature of "fat hull" material in milk has been the subject of numerous investigations since 1840, and divergent conclusions have been reached. The material has been variously reported as casein (31), glyco-

protein (30), a mixture of globulin-like proteins and phospholipids and so forth. Palmer and Wiese (32) in a recent publication point out that the material consists of a mixture of proteins and phospholipids, and that the protein possesses the properties of both a hydrophyllic and a hydrophobic colloid. They point out further that its physical properties, percentages of N, S, and P, do not correspond with any of the other milk proteins. It

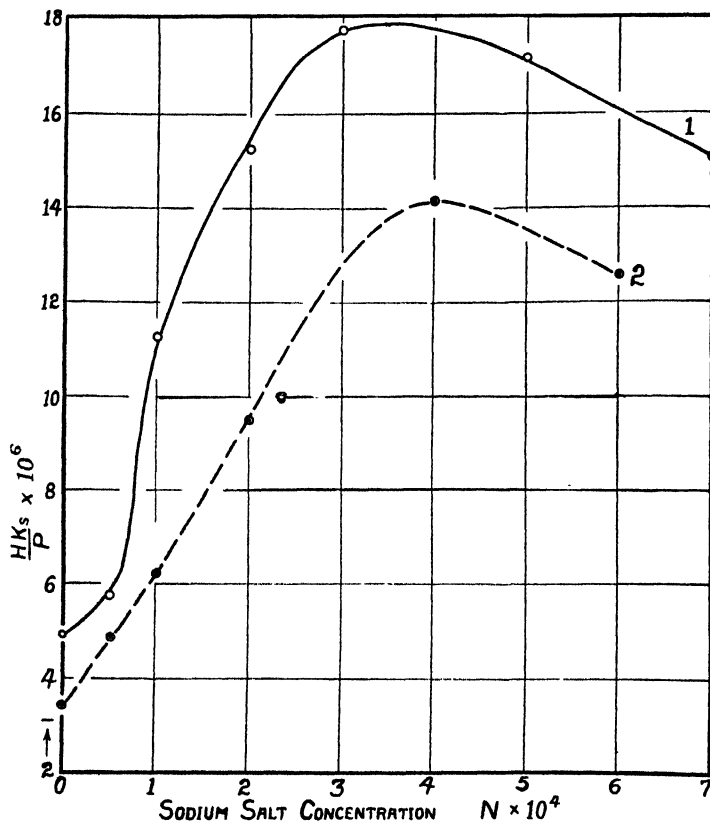


FIGURE 6  
1 = Sodium Citrate  
2 =  $Na_2HPO_4$

should be pointed out, however, that their comparisons of S, N, and P contents were with analytical data taken from the literature rather than with analyses of milk proteins that had been subjected to the same special treatment that they employed in preparing the "membrane" protein. Nor do they consider the data of Titus, Sommer and Hart (31) which shows good agreement between the "membrane" protein and casein with respect to

nitrogen distribution, content of sulphur, phosphorus and tryptophane, specific rotation, and the precipitin reaction. The latter investigators concluded that the "hull" material consisted chiefly of casein.

For the present purpose it is sufficient to consider that there is a protein adsorbed at the fat-serum interface, and that this must necessarily alter the conditions with respect to the electrokinetic potential. Similarly, adsorbed phospholipids, postulated by Palmer and Samuelson (24), must exert an effect. That the adsorbed material alters the potential is shown by table 2, which shows that the electrokinetic potential is lower (less negative) after

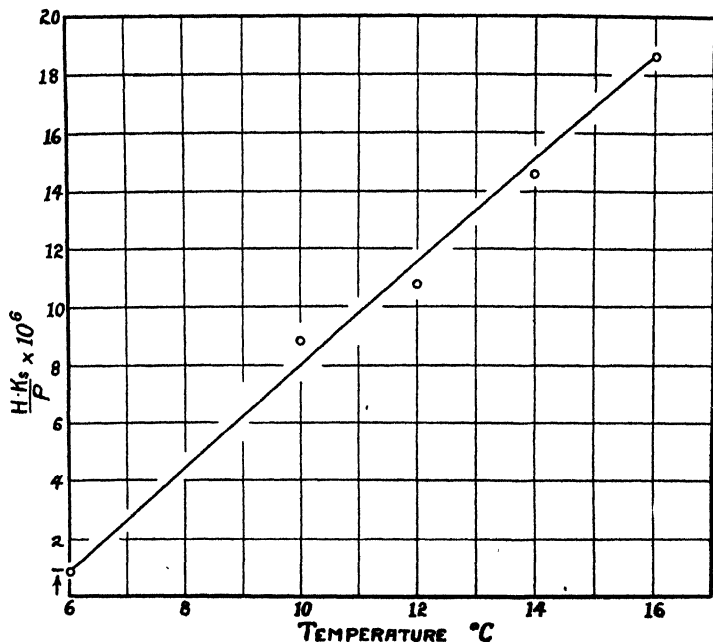


FIGURE 7

opportunity and time have been provided for adsorption. The work of Prieger (27) shows an isoelectric point of pH 2.27–3.07 for fat globules suspended in water, and pH 4.21 in milk. Since such reactions are on the acid side of the iso-electric point as commonly found for milk proteins (pH 4.6–4.7), the proteins by themselves should have a positive potential. Such proteins adsorbed on the fat globules should bring the iso-electric point of the globule nearer to pH 4.6. In the present work the iso-electric point or the point where the potential at the fat-serum interface is zero was found to be pH 4.3 (Table 4 and Figure 4).

The potential at the interface must be the net result of adsorbed ions and special properties conferred by irreversibly adsorbed material. The

TABLE 11  
Effect of temperature

° C.	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{**}$
	<i>mm.</i>	<i>millivolts</i>		<i>Mhos</i>	<i>(-)</i>
6.0	147	175	1.1	0.90	0.99
	180	200	1.1		
	220	250	1.1		
8.0	147	470	2.6	1.60	2.86
	180	600	2.7		
	220	740	2.6		
10.0	147	750	5.0	1.82	8.92
	180	900	5.0		
	220	1050	4.7		
12.0	147	810	5.5	2.2	10.8
	180	975	5.4		
	220	1200	5.4		
14.0	147			2.18	14.60
	180	1200	6.6		
	220	1500	6.8		
16.0	147	1225	8.3	2.24	18.59
	180	1500	8.3		
	220				

special properties contributed by the irreversibly adsorbed materials involve adsorption of ions by it and the dissociation of the irreversibly adsorbed material itself. Since proteins are ampholytes, the following conditions apply with respect to reaction:—

Protein dissociated as a base, therefore the potential will be progressively more positive as the acidity is increased

(B)

← Increasing  
Acidity

Iso-electric  
Protein

→ Decreasing  
Acidity

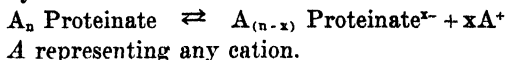
Protein dissociated as an acid, therefore the potential will be progressively more negative as the acidity is decreased or the alkalinity is increased

(A)

This reaction must be expected to affect the potential because (1) it fixes the hydrogen- and hydroxyl-ion concentrations and their direct absorption (2) it determines the type and extent of dissociation of the irreversibly adsorbed protein, and (3) it determines the degree of dissociation and valence of polyvalent ions such as citrate and phosphate ions.

The ions furnished by salts must influence the potential by (1) the direct adsorption of the ions, and (2) the effect of the ions on the dissociation of the adsorbed protein. Under (A) above the proteins yield compounds such as sodium proteinate, potassium proteinate, etc. The extent

to which these compounds are dissociated must, in conformity with the mass law, be affected by the concentration of the cations. Thus:



The application of the mass law to this problem cannot be made in a quantitative manner, but in a qualitative sense it must be applicable. Any complete explanation, no doubt, must involve a number of additional factors, such as the effect of double bonds and natural salt or ketopiperazine structures within the protein molecule.

The effect of added salts was in general in harmony with expectations. The chlorides (with the exception of KCl which first increased and then decreased the potential) all decreased the electrokinetic potential. Since in these salts the chloride ion is common to all, the cation should, therefore, be the effective ion, the efficiency increasing with the valence. In the case of the Thorium ion which is tetra-valent, the charge was actually reversed.

The phosphate ion was slightly less effective than the citrate ion in making the charge more strongly negative. This is to be expected from the dissociation constants of the phosphoric acid and the citric acid, which are as follows: phosphoric acid,  $k_1 = 1.1 \times 10^{-2}$ ,  $k_2 = 1.95 \times 10^{-7}$ ,  $k_3 = 3.6 \times 10^{-13}$ ; citric acid,  $k_1 = 8.3 \times 10^{-4}$ ,  $k_2 = 4.1 \times 10^{-5}$ ,  $k_3 = 3.2 \times 10^{-6}$ . The titration curves of phosphoric and citric acid, fixed by these constants, show that at reactions involved in these and similar studies, the citric acid is more completely neutralized than the phosphoric acid. For example at pH of 6.5, soluble phosphates exist in the proportion of 3.12 moles of primary phosphate to 1.95 moles of secondary phosphate. In considering the effect of added phosphates at this reaction (even if the addition had been as tertiary phosphate,  $(Na_3PO_4)$  the phosphate ion should, therefore, be considered partly mono-valent and partly di-valent. A similar calculation for citrates can not be given with the same degree of accuracy because the three dissociation constants for citric acid are more nearly of the same value; but inspection of the dissociation constants and the titration curve for citric acid, clearly shows that the citrates will exist partly as the di-basic and partly as the tri-basic salt at pH 6.5. These facts are pointed out in some detail, because the error is frequently made of considering citrates and phosphates as furnishing tri-valent ions regardless of the reactions.

Temperature is known to affect the dissociation of acids, bases, salts and of water itself, and the activity of the ions. It is therefore to be expected that temperature should affect the electrokinetic potential. As is shown in table 11, increasing the temperature increases the negative potential quite rapidly. In a complex system such as milk it is difficult to attribute this effect to a single factor or several specific factors; however, it is likely that the following considerations are involved: (1) Since hydroxyl ions are more strongly adsorbed than hydrogen ions, the increased

dissociation of water with higher temperatures should favor the greater adsorption of hydroxyl ions. (2) Higher temperatures are likely to increase the dissociation of the adsorbed protein as an acid. (3) The increased dissociation of the salts with increased temperatures is likely to be most significant with the citrates and phosphates, especially the latter; increased dissociation in this case would increase the valence of the ions (*e.g.*, phosphate ions changed in the direction of primary to secondary to tertiary). (4) Increased temperatures decrease the hydration of the irreversibly adsorbed proteins thereby moving the boundary which is involved in the electrokinetic potential closer to the globule itself.

The factors which are shown to affect the electrokinetic potential at the fat-serum interface must also be expected to affect the potential or charge on the colloidal protein particles themselves. This must follow if the adsorbed proteins play the dominant rôle in determining the charge on the fat globules. As far as can be stated at the present time, the effects of salts on the stability of milk proteins, as reported by Sommer and Hart (29), are in harmony with this expectation. It is further supported by the work of Doan (4) (5) (6) which shows that there is a relationship between the clumping of fat globules and the stability of proteins

#### SUMMARY

1. A method is described, based on the theory of streaming potentials, that may be used in measuring the magnitude of the electric charge at the fat-serum interface.

2. The stability of colloidal systems is discussed and a mathematical development of the streaming potential formula is given.

3. Data are presented to show that an interface quite comparable to that of the fat globule as it exists in milk is being considered.

4. The electrokinetic potentials observed using various samples of milk from individual cows are shown to vary over a comparatively wide range.

5. The iso-electric point of the interface was found to be at about pH 4.3.

6. Potassium chloride, added in increasing amounts, caused an increase that was later followed by a decrease in potential. Chlorides of calcium, iron and thorium decreased the electrokinetic potential. Thorium was found to be the most effective and at certain concentrations reversed the sign of the charge.

7. Di-sodium phosphate and sodium citrate caused the potential to become more strongly negative. The latter salt was found to be the most effective.

8. Increasing temperatures produced a very pronounced increase in the observed electrokinetic potential.

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# X-RAY INVESTIGATION OF THE MICROCRYSTALLINE STRUCTURE OF BUTTER-FAT

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It may be expected that one of the factors which have an influence on the firmness of butter will be the way in which and to what extent butter-fat crystallizes. A far advanced crystallization can be obtained by holding the cooled cream before churning, or the butter for a long time. By following the first method the fresh butter will be much more solid than butter made without cooling the cream beforehand. As a result hereof it can be worked more and drier and will give a finer distribution of the liquid; moreover, less fat will get lost in the buttermilk. Although it may be assumed that in this butter the fat is practically in equilibrium at the churning temperature, the butter still strongly solidifies when kept at the same temperature, which behavior, therefore, cannot be ascribed to a further crystallization. It is, however, not impossible that a variation in the size of the crystals is the cause of this phenomenon, or that some other change in microstructure occurs. Also for other plastic substances this question seems not yet to be solved.

The cooling of butter prepared from cream that has not been held cooled beforehand differs from the cooling of butter from chilled cream. At first, the fat of this butter is not in equilibrium as the greater part is still in the non-crystallized state. on cooling, this part will slowly solidify in the butter with a chance of forming crystallites of another size. Thus, in this case, the way in which the crystallization takes place and which we discussed above, would be different from that which occurs in the first mentioned butter. When, by following the two methods indicated, summer-butter is prepared from the same cream and the butters are kept at a temperature of about 9° C., then the original great difference in firmness almost completely disappeared after a week; it even happens that the product, which was softer when fresh, turns out to be the more solid one later on. Yet in most cases the body of the butter, prepared from cream that was cooled beforehand, is better, as it proves to be more "elastic"; the other sort, on the contrary, is not elastic, especially not when the churning temperature has been rather high.

The size of the fat-crystallites can be influenced also in a different way, namely by varying considerably the temperature of the washing-water. When this temperature is chosen considerably lower than the churning tem-

<sup>1</sup> The X-ray part of the investigation.

perature, *i.e.*, lower than about  $6^{\circ}\text{C.}$ ,<sup>2</sup> then a rapid crystallization will take place, since the fine butter grains will also easily assume this temperature. When, however, water is used, which is but  $1$  or  $2^{\circ}$  colder than the churned product and the butter is cooled afterwards, then the cooling of the butter will take place slowly again, thus causing another way of crystallization. When following this method, the difference in structure can be seen very distinctly on the fracture with the unaided eye. The butter rinsed with very cold water shows a fine texture, the other a coarse, more normal structure.

From the aforesaid it becomes evident that a more exact knowledge of the microcrystalline structure of butter-fat would be very important in practice and this is the reason why we have endeavored to investigate to what extent an X-ray investigation would be of use. Although our experiments in this direction have not yielded the results we expected from them, we thought it useful to present them.

Some general remarks as to the method of procedure which was followed for the X-ray investigation may be given first.

X-ray interference exposures were made of all sorts of butter-fat to be investigated according to the method of Debye-Scherrer. To this end the material (see fig. 1)<sup>3</sup> is fastened in the form of a thin rod P to the axis of

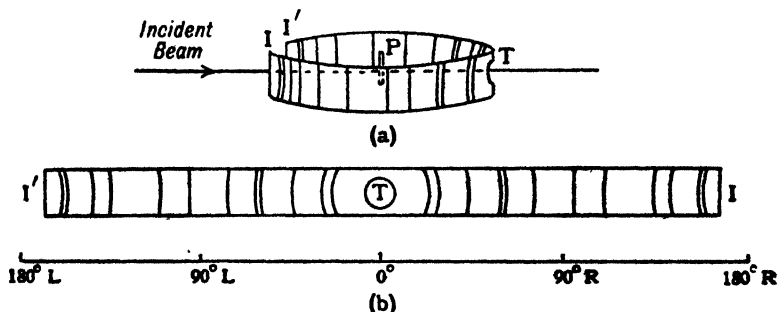


FIGURE 1

a cylindrical camera and exposed to a narrow beam of monochromatic X-rays, which enters the camera through a tubulated diaphragm in the wall. In case of microcrystalline material, containing a large number of crystallites with arbitrary position of their axes with regard to the incident X-ray beam (as will be generally the case in any crystal powder), the scattered rays, due to interference of the incident beam with the crystal lattice planes, form a system of circular cones round this beam as an axis. These cones intersect a film I-I', which is placed along the wall of the

<sup>2</sup> Hildeah. Molkerei. Zeit. 43: 1198, 1929.

<sup>3</sup> From: W. L. Bragg, The Crystalline State, London, Bell & Sons, 1933, where a modern exposition is given of the theory and the method of X-ray investigation.

cylindrical camera, according to curved lines which, in first approximation, form a system of concentric circles (in general, as a consequence of the limited height of the film, circular *arcs*) with the point T, where the incident beam of X-rays strikes the film, as the common center. From the distance of the circles and the diameter of the camera, the top angle,  $4\theta$ , can be calculated for each cone and from this, knowing the wave length  $\lambda$  of the X-ray light used, by means of Bragg's well-known formula  $\lambda = 2d \sin \theta$ , the distance of periodicity  $d$  of the crystal lattice planes, corresponding with each special circle.

From the character of the interference lines, data concerning the size of the crystallites can be derived in certain cases: when this size is larger than about  $10\mu$ , the lines are no more continually blackened, but the rays scattered by the individual crystallites give rise to separate points on the circles; when, however, the crystallites are smaller than  $0.1\mu$ , the lines will be considerably broadened as a result of insufficient resolving power.

Finally it may be remarked that a liquid phase gives rise to one or more very diffuse interference circles (halos) with a diameter related to the mean distance of the molecules in the liquid, which latter, as a result of thermal agitation, varies constantly.

The photographs reproduced hereafter were obtained with the Philips Metalix apparatus for the investigation of crystal structure.<sup>4</sup> To obtain a preparation of the butter, suitable for an exposure, a capillary tube (total diameter  $\frac{1}{2}$ –1 mm) with thin walls, for X-rays transparent glass, is dipped several times in the butter, till it contains a sufficient quantity. In order to prevent the temperature of the butter fat to be influenced by this manipulation, especially in view of the very small quantity required for an exposure, the capillary was kept close to the butter beforehand, so that both had the same temperature; then it was taken up with cottonwool or gloves to reduce warming by the hand. The preparations were then put in the center of a Debye-Scherrer camera (diameter 57.3 mm) belonging to the above-mentioned apparatus for crystal structure and exposed during half an hour with copper-K $\alpha$ -radiation ( $\lambda = 1.54 \text{ \AA}$ ). A thermometer was entered through an opening in the cover of this camera, the reservoir close to the preparation, so that the temperature of the latter could be controlled during the exposure. In special cases, where it was not allowed to surpass a certain maximum temperature, cooled air was blown through side tubes, mounted in two diametrically opposite points of the camera wall, with such velocity that the thermometer indicated the desired temperature.

*1st Experiment: Crystallized butter-fat in comparison with butter.*

After some preliminary experiments, exposures were made of butter-fat of very high and of very low melting point in comparison with ordinary

<sup>4</sup> A. Bouwers and W. Busse, ZS. f. Krist. 77, 507, 1931.

butter as such. In order to obtain the two first sorts, a liquid part was separated at 11° C. from filtered butter-fat, the solid rest being melted and then crystallized at about 32° C.; then the crystals were filtered and washed with a mixture of alcohol-petroleum ether, the rests of which are removed from the crystals by introducing dry carbonic acid in the molten mass at a mercury pressure of about 5 mm.

The resulting X-ray photographs are reproduced in fig. 2 *A*, *B* and *C*.

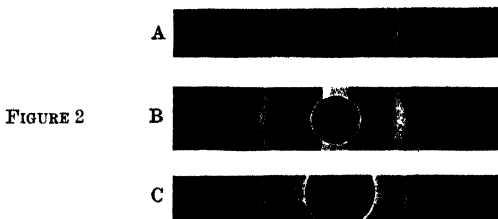


FIGURE 3



*A* relates to the fat with low melting point ("liquid oil"), *C*, to that with a high melting point ("solid fat"), whereas *B* represents an exposure of ordinary butter at about 18° C. The photographs are in agreement with what might be expected from the above given general remarks: *A* shows one diffuse circle (halo), which is characteristic for a liquid phase, *C* some (2 are clearly visible) relatively sharp interference lines, as can be expected for a solid crystalline material.

In connection with the known diameter of the camera and the wave length of the X-ray radiation used, it is found from the distance of the lines that these correspond with periodicity distances in the crystal lattice of resp. 4.32 and 3.87 Å, that are distances of the order of magnitude as are generally found for the "cross-dimensions" of molecules with long chains of carbon atoms.<sup>5</sup> Photograph *B* of the butter preparation can be considered as a superposition of the photographs *A* and *C*; it shows the "oil-ring" as well as the "solid crystal lines" (only the outmost of these two is

<sup>5</sup> See e.g. ZS. f. Krist.: Strukturbericht 1913-1928; the much larger periodicity distance, which corresponds with the *length* dimensions of the fat molecules, gives rise to an interference cone with very small top angle. In connection with the dimensions of the camera, this cone cuts the film along so small a circle that the latter falls within the opening in the middle of the film, through which the direct beam leaves the camera. Consequently this interference circle cannot appear on the film.

visible separately, as the inmost line just coincides with the exterior part of the oilring; compare also fig. 3). *Thus the photograph shows very distinctly the presence of liquid fat as well as of solid fat in the butter.*

FIGURE 4



FIGURE 5



*2nd Experiment: Influence of keeping butter at low temperature on its crystallization.*

The occurrence of the "oilring" in experiment 1 led to the next experiment.

A portion of summer-butter, prepared from cream, which had not been cooled to a very low temperature, was exposed immediately after preparation (fig. 3 A), whereas another portion was kept during two months at a temperature of  $-10^{\circ}$  to  $-12^{\circ}$ , after which a photograph was made (fig. 3 B).

A comparison of these two photographs shows very distinctly that, as a result of keeping the butter at low temperature during a long time, a strong crystallization of solid butter fat has taken place; the "oilring," still distinctly visible on photo 3 A,<sup>6</sup> has almost completely disappeared on photo 3 B, and has been replaced by the two "crystal-lines." From the fact that these lines, although close together on the film,<sup>7</sup> differ apparently considerably in width, it seems probable that line-broadening as a consequence of the smallness of the crystallites occurs and that these crystallites, at least as regards one of their cross-dimensions, are smaller than  $0.1\mu$ .

*3rd Experiment: Influence of wash-water of a very low temperature in comparison with that of normal temperature on the crystallization of butter.*

Two equal portions of sour cream (cooled after pasteurization at about  $9^{\circ}$  C., soured at  $13^{\circ}$  C.) were churned in March and one portion of the butter washed with very cold water, the other in the normal way:

<sup>6</sup> Unfortunately the left side of this photograph has been damaged.

<sup>7</sup> As, namely, the line broadening is dependent on the reflection angle  $\theta$ , only lines which lie close together on the film (thus differing little in  $\theta$ ) may be compared with each other.

	Cold rinsing	Warm rinsing
churning temperature . . . . .	13.3°–14.5°	13.3°–14.5°
duration of churning . . . . .	32'	35'
temperature washing-water . . . . .	3.6°	13°
temp. butter after first working . . . . .	6.8°	13.2°
temp. butter after second working . . . . .	9°	12.8°
number of revolutions . . . . .	36	18

That same day Debye-Scherrer exposures were made of both sorts of butter (fig. 4 *A* and *B*) and this was repeated after they had been kept during a week at a temperature of 8° (fig. 5 *A* and *B*). After this week the consistency, which, of course, considerably differed in fresh condition, proved to be almost equal after heating up to 17° C.; with the Perkins-apparatus the following results were obtained for hardness:

"cold rinsing," at 16.8° C. . . . .	0.23
"warm rinsing," at 16.7° C. . . . .	0.24

The difference in structure mentioned above was very well visible on the fracture and the coldly washed product showed the well-known appearance of solidified limiment.

Inspection of the photographs does not show a marked difference between the two sorts of butter, neither in fresh condition nor after a week's storage at 8°. Although the method of exposure is not very "sensitive" with regard to the detection of small differences in size of the crystallites, it can yet be concluded from this fact, that the size of the crystals in the two kinds of butter does not differ considerably and consequently it does not seem very likely that the marked difference in structure must be ascribed exclusively to a difference in crystal size. However, it must be kept in mind that, as indicated already above, the interference circle due to the periodicity distance of the crystal lattice corresponding with the *length*-dimension of the fat-molecules, is *not* present on the photographs: the possibility thus remains that the dimensions of the crystallites parallel to the length-direction of the molecule show larger differences (so that they would give rise to interference circles which differ distinctly in sharpness).

Finally it may be remarked that an eventual marked anisodiametrical form of the fat-crystallites (rods or plates) may give rise to "performance orientations" during the working. The fact that preference orientations can occur when spreading fat is proved by experiments of J. J. Trillat.<sup>8</sup> A difference in "habitus" of the crystallites is in all probability accompanied by a difference in "orientation power" and might eventually come to expression as a difference in external structure of in elasticity.

<sup>8</sup> J. J. Trillat, C. R. de l'Acad. d. Sc., 182, 843, 1926; Ann. d. Physique [10] 6, 5, 1927; see also: "Les applications des Rayons-X" (Les Presses Universitaires de France, Paris, 193).

## A CHART TO AID IN SCORING MILK FLAVOR

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In the summer of 1933, a project was inaugurated at the Oklahoma station to study certain phases of the question of flavors in milk. At the outset it was recognized that work of this nature depended to a considerable extent on the accuracy of the scores accorded the milk samples. It was further realized that the scoring of milk can not give wholly exact and uniform results but is subject to considerable variation among individual judges. Even a given judge, though entirely proficient, is by no means infallible in such a delicate matter as the evaluation of milk flavor.

In contemplating the procedure to be employed in obtaining the milk scores in this project two alternatives presented themselves. On one hand, there was the possibility of using as judges two of the staff members who had acquired considerable experience in scoring and judging work. On the other hand, there were four additional staff members available who could assist the two experienced men.

It was finally decided the use of all six men would be the more suitable procedure in such a study. There appeared to be considerable advantage from the standpoint of accuracy, in securing the greater number of individual scores. Also the greater number of persons whose scores were secured could more nearly represent the view-point of consumers. The significance of this view-point is not to be minimized in studies involving milk quality.

On starting the work there immediately arose the need for some device to aid, especially the inexperienced men, in scoring the milk for flavor. These men sought first of all a proper vocabulary so they could reveal the flavors they detected. They as well as the experienced judges wanted some guidance as to the seriousness of each flavor defect. To accomplish these objectives the chart as shown here was prepared and displayed in the laboratory where the scoring was done.

Efforts in formulating guides in scoring milk for flavor have been made by other workers. Babcock and Leete<sup>1</sup> proposed a "general guide for scoring flavor and odor." Lucas<sup>2</sup> also has suggested certain numerical scores for various flavor defects in milk.

The rules for the students' National contest in judging dairy products carry a detailed milk score card in which 15 flavor defects are listed. This

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<sup>1</sup> Babcock, C. J., and Leete, C. S. How to conduct milk and cream contests. U. S. Dept. Agr. Cir. 384: 18. 1929.

<sup>2</sup> Lucas, P. S. Many factors cause abnormal milk flavors. Mich. Exp. Sta. Quar. Bul. XII: 1. 1929.

material while providing terms that may be used to describe a defect affords no guidance as to the score that should be accorded when the defect varies in intensity.

#### THE PROPOSED CHART

In the chart here are included the five classes into which milk is divided on a basis of flavor as suggested by Babcock and Leete. The perfect score for flavor is 25. Milk that scores 23 or above, is designated as *excellent*, 21 or 22 *good*, 18, 19 or 20 *fair*, 12 to 17 inclusive *poor*, and 11 or below is *bad*. A fractional score in no case serves to raise a sample into a higher class than it would enter without the fraction. For instance, a sample scoring  $20\frac{1}{2}$  is classed as fair, not as good. Eighteen different defects in flavor are enumer-

#### Flavor Defects and Suggested Scores

(s. indicates slight; v. indicates very)

CLASS	EXCEL- LENT	GOOD	FAIR	POOR	BAD
SCORES	23 and above	21 and 22	18, 19 and 20	12 to 17, incl.	11 and below
Acid				s. acid	acid v. acid
Bitter				s. bitter and bitter	v. bitter
Cooked Cow		s. cooked s. cowy	cooked cows	v. cooked v. cowy	
Disinfectant				s. disinfectant	disinfectant and v. disinfectant
Feed		s. feed	feed	v. feed	
Flat		s. flat	flat	v. flat	
Metallic			s. metallic	metallic and v. metallic	
Musty				s. musty	musty and v. musty
Nutty			s. nutty	nutty and v. nutty	
Oxidized			s. oxidized	oxidized and v. oxidized	
Rancid				s. rancid	rancid and v. rancid
Salty			s. salty	salty	v. salty
Sharp		s. sharp	sharp	v. sharp	
Stale			s. stale	stale and v. stale	
Sweet		s. sweet and sweet	v. sweet		
Watered			s. watered	watered and v. watered	
Weedy				s. weedy	weedy and v. weedy

ated. It is recognized that some of these terms are subject to challenge. Again some judges would choose to add other terms. In a year's work with this chart, during which time about 1,000 milk samples have been scored by five or six judges, a few suspected off-flavors other than the eighteen enumerated have been encountered. However, none of these other flavors

has been observed with enough certainty or frequency to justify its inclusion in the chart.

In formulating this chart it was believed each flavor could be resolved into at least three degrees of intensity. Accordingly the terms "slight" and "very" are employed to reveal the upper and lower gradations in the flavor. These terms, or comparable ones, are used by all judges. The effort in the chart is to ascribe some numerical significance to the different gradations.

It is observed in several cases here that two gradations of a given flavor are suggested under the same class. For instance, milk that is either "acid" or "very acid" is classed as *bad*. Again, milk that is "slight bitter" is held down into the same class as "bitter" milk. In devising the chart decisions that relegated the different flavor gradations into the various classes were based on experience and usual practice in scoring milk. They are entirely arbitrary and no effort is made here to defend them. On the whole, however, it is believed few workers would have occasion to deviate greatly from the suggestions as given.

Frequently in using this chart the judges have found occasions which prompted them to use an additional gradation to reveal a "very slight" intensity in some flavor. Such occasions have arisen most frequently with the salty samples. It is suggested in the chart that milk found to be "slight" salt should be classed as *fair*. However, the judges sometimes detected a very slight taste of salt that was in no wise objectionable. They felt it was unduly critical to hold the milk down to *fair* so used the gradation "very slight" salt and kept the milk in the *good* class.

#### OBSERVED BENEFITS IN USING THE CHART

In the year's use of the chart during which time about 1,000 samples have been scored by five or six judges some observations thought to be of value have been made.

1. The use of the chart contributes somewhat to the unification of the scores accorded by individual judges. Of course, it does not make for anything like perfect unanimity. Variation in the scores will exist unless the judges confer and coordinate their criticisms of each sample.

2. In work of this nature involving studies of milk quality, the procedure wherein several judges, under such guidance as the chart provides, express their individual opinions is believed preferable to the plan wherein the judges confer to attain greater uniformity in the scores. The former plan can be related to the consumers' view-point more directly than the latter.

3. The use of the chart provides an incentive for the judges to score inferior milk sufficiently low so it is effectively differentiated from superior milk. It is believed that usual practice in scoring milk is characterized by

an unduly critical attitude toward superior milk and undue liberality toward inferior milk.

4. Such a chart fortifies one's vocabulary so he feels greater security and confidence in scoring milk.

5. It affords, not only amateurs but experienced judges as well, some guidance as to the seriousness of various flavor defects in milk. The recognition of the five classes of milk based on flavor, namely; excellent, good, fair, poor and bad seems particularly helpful.

6. The chart is conducive to a "flavor consciousness" among men who are interested in problems relating to milk, such as college and short course students and plant men.

7. It proves somewhat fascinating because it depicts graphically information desired by men of the types named above, most of whom are eager to acquire some proficiency in scoring milk.

8. The use of the chart has proved an asset in giving instruction in milk judging.

## THE EFFECT OF ALFALFA HAY ON MILK FLAVOR

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Many workers have called attention to the fact that the feed a cow receives may have a pronounced effect on the flavor of the milk. Particular attention has been directed toward the dangers in using the succulent feeds. Peculiarly enough the injury to milk flavor that is caused by alfalfa hay seems in many cases to have escaped notice. Some investigators, in ascertaining the effects of various succulent feeds on milk flavor, have even used alfalfa hay in the check rations apparently in the belief the hay exerted no effect. On the other hand, Roadhouse, Regan and Mead (1), in 1926, recognized that alfalfa hay exerted an effect on milk flavor. They mentioned its effect was less pronounced than when the alfalfa was used as pasture or silage. Also Lucas (2), in 1929, said, "Alfalfa hay gives to milk a rather pronounced flavor but it is objected to by only a very few people."

Probably others both in research work and dairy practise have observed the effects of alfalfa hay on milk flavor but like Lucas considered these effects of little consequence or in a desire to avoid any criticism of alfalfa as a dairy feed deliberately omitted comment on their observations.

The interest of the authors in ascertaining the effect of alfalfa hay on milk flavor was prompted by two incidents. One of the better milk plants in Oklahoma had during 1931 encountered feed flavors, the source of which seemed to be alfalfa hay. Reluctance was felt in granting that this feed might cause as serious defects as were observed but a study of the question seemed justified.

At about the same time there was under way at this station a feeding trial in which mungbean hay was compared with alfalfa hay to determine their values in milk and butterfat production. Churnings were made of cream produced on each feed to determine the effect of the mungbeans on the quality of the butter. It was observed that the butter from the alfalfa hay rations carried a distinct feed flavor while that from mungbean hay failed to show the flavor and invariably received higher scores.

### PROCEDURE

During the winter of 1933-34, we conducted a trial to determine the effect upon milk flavor of varying quantities of alfalfa hay fed at varying time intervals before milking. Twelve cows were used. They were kept in adjoining experimental stanchions except for five or six hours daily when they were kept in a dry lot. They were fed alfalfa hay and a concentrate

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mixture consisting, by weight, of 4 parts corn, 2 parts oats, 2 parts bran and 1 part cottonseed meal. Some of the cows received no succulent feeds, others received mangels and others darso silage.

The alfalfa hay used was grown locally and was fed from the bale. It was described as No. 1, leafy, fine, quite green and free from foreign matter. The interval before milking at which the hay was fed varied from one-half to seven hours. In this work involving the time interval four pounds of hay was always used as a standard quantity because it was about the proper amount for one feeding for the smaller cows weighing approximately 800 pounds. In the work wherein the quantity of hay was varied two, four and six pound quantities were fed at a uniform interval of one hour before milking.

The concentrate mixture was fed all cows just before the first cow in the string was milked. The mangels or silage was fed each cow immediately after she was milked.

The cows were milked twice daily by one man who seldom varied by as much as two minutes from his scheduled time for each cow. This close adherence to the milking schedule made it possible to devise a hay feeding schedule to provide the desired interval between feeding and milking.

The work on this problem was restricted entirely to the evening milkings which started at 3:45 P. M. As each cow was milked her milk was carried to the milk room. A sample was strained into a 10-ounce bottle. The bottle was capped and cooled down in tap water with frequent shaking to about 50° F. When all cows were finished at about 5:25 P. M., the samples were removed from the water and placed in the refrigerator at 40° F. till the next day at 8:00 A. M. when they were heated to about 95° F. and scored for flavor. The samples, identified only by number, were scored by six members of the staff. On the score card for milk 25 points are allowed for a perfect flavor.

## RESULTS

### *The Interval Between Feeding and Milking*

In this work, 12 samples from each cow were scored by six staff members which gave 72 observations on each cow or a total of 864 observations on the entire 12 cows. In table 1 are shown the average scores on all cows by individual judges and in the right-hand column the composite scores by all the judges.

The data in table 1 reveal considerable uniformity among the staff members in scoring the samples. Without exception the check samples—in the first line when the cows were fed hay after milking—scored highest. With four of the judges the milk collected after the 2-hour interval scored lowest. All judges' scores were quite consistently higher as the interval was prolonged beyond 2 hours.

TABLE 1  
*Scores by individual judges on all the cows' milk when alfalfa was fed*

INTERVAL BEFORE MILKING	JUDGE'S INITIAL						ALL JUDGES
	F	Ku	W	M	Ke	R	
Fed after	21.9	22.2	22.3	21.9	21.9	21.4	21.9
$\frac{1}{2}$ hour	19.9	19.0	19.9	19.7	19.4	absent	19.6
1 hour	18.8	18.3	18.5	18.5	19.2	absent	18.7
2 hours	18.3	17.9	18.9	17.7	19.0	18.9	18.5
3 hours	19.3	18.8	19.8	19.0	19.5	18.9	19.2
4 hours	20.0	19.9	21.0	20.8	19.8	20.9	20.4
5 hours	21.2	21.0	22.0	21.6	20.3	21.6	21.3
6 hours	21.2	21.6	21.7	21.5	20.9	21.1	21.3
7 hours	21.1	21.9	21.8	21.5	20.6	21.0	21.3

The feeding of four pounds of hay one-half hour before milking decreased the scores from 21.9 to 19.6. The milk flavor was seriously affected. The 2-hour interval gave the lowest scores of all. This observation is in agreement with that of Roadhouse, Regan and Mead. Alfalfa hay exerts its worst effect if fed at this interval. Beyond the 2-hour interval the scores increased to 21.3 at the 5-hour interval and remained at that point for the 6- and 7-hour intervals.

The failure of the scores after the longer intervals to regain the level of the check samples is noticeable. In scoring these samples the judges were

TABLE 2  
*Scores by all judges on milks of individual Holsteins when alfalfa was fed*

INTERVAL BEFORE MILKING	COW'S NUMBER				ALL FOUR HOLSTEINS
	1	2	3	4	
Fed after	22.6	22.1	22.3	21.6	22.2
$\frac{1}{2}$ hour	21.0	22.4	20.4	20.6	21.1
1 hour	21.2	21.0	19.0	18.8	20.0
2 hours	21.2	21.3	19.5	18.7	20.8
3 hours	20.5	22.2	19.2	21.0	20.7
4 hours	21.8	22.3	22.3	22.0	22.1
5 hours	21.0	21.8	21.2	22.6	21.7
6 hours	21.7	21.7	22.8	21.6	22.0
7 hours	21.8	21.7	22.4	21.8	21.9

extremely alert to any indication of a feed flavor. With some of the cows the effect of the alfalfa hay was entirely eliminated when fed four hours before milking. With other cows a slight off-flavor persisted in milk drawn even as long as seven hours after feeding. However this slight flavor was scarcely discernible; only a very small percentage of persons would detect it.

When the first scoring was done it became apparent that the large Holstein cows in the trial showed far less effect from the four pounds of hay than the smaller Jerseys. There were four purebred Holsteins and three purebred Jerseys. The remaining five cows in the trial consisted of two purebred Guernseys and three grades intermediate in body weight between the purebred Holsteins and Jerseys.

Table 2 shows the response of the Holsteins to the feeding of the hay. Table 3 gives corresponding data for the Jerseys.

TABLE 3  
*Scores by all judges on milks of individual Jerseys when alfalfa was fed*

INTERVAL BEFORE MILKING	COW'S NUMBER			ALL THREE JERSEYS
	8	9	10	
Fed after	22.0	22.6	22.1	22.2
$\frac{1}{2}$ hour	18.2	18.2	17.8	18.1
1 hour	19.0	17.6	16.4	17.7
2 hours	17.2	16.0	16.8	16.7
3 hours	17.0	17.7	17.0	17.2
4 hours	18.0	20.3	18.2	18.8
5 hours	22.8	20.5	19.5	20.9
6 hours	21.2	21.5	20.8	21.2
7 hours	22.5	21.8	21.0	21.8

From tables 2 and 3 it is observed that the check samples from the Holsteins and the Jerseys scored exactly the same, 22.2 points. After the one-half hour interval the Holstein scores dropped only 1.1 points to 21.1. The Jerseys dropped 4.1 points to 18.1. Also the Holsteins showed the lowest score after the 1 hour interval while the Jerseys showed the lowest score after the 2 hour interval. With the Holsteins the milk obtained after the 4 hour interval or later was scored practically as high as the check samples, while the Jerseys still showed a slight effect on their milk even after the 7 hour interval. The flavor score of the milk from the Holsteins never dropped below 20.0. That of the Jerseys went as low as 16.7.

In the absence of a definite explanation of the difference in behavior between the two breeds, the conjecture is offered that this difference is

largely a matter of body weight. The four pounds of hay for the 1400-pound Holsteins is relatively less significant than for the 800-pound Jerseys. On the other hand, some additional factors may be involved. It is possible the Jersey milk because of its higher fat percentage carries more of the feed flavor than Holstein milk. Also there is the possibility the Jersey naturally produces milk with a higher flavor. Further attention is being devoted to this particular phase of the problem.

#### AERATION AND COOLING

In such a problem as this involving a study of milk flavor interest arises in the possibility of removing the flavor by aeration or cooling or both procedures. Some study has been directed along these lines. Cooling without aeration apparently was ineffective in removing the flavor. Aeration alone accomplished fully as much benefit as combined aeration and cooling, but even the aeration did not remove all the flavor.

It has been possible by aeration as well as by combined aeration and cooling to secure milk which scores about mid-way between the checks—those having no feed flavor—and the feed-flavored milk that was neither aerated nor cooled. In other words, the aeration removes about one-half the flavor imparted to milk by the hay.

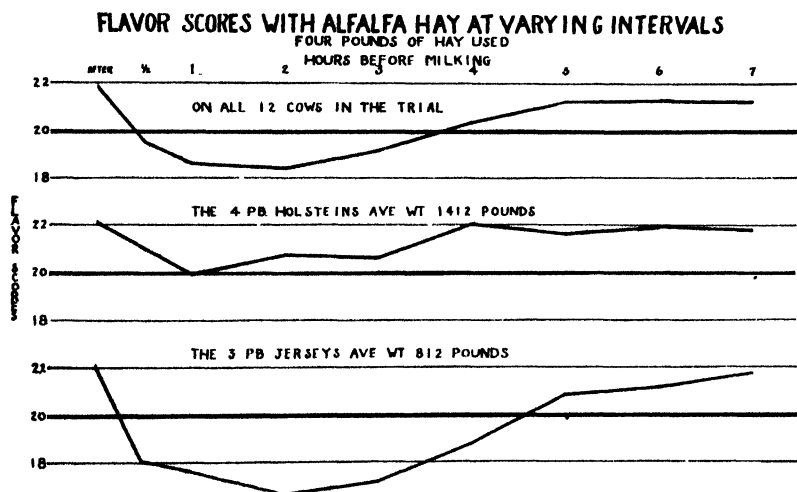


FIG. 1. EFFECT ON MILK FLAVOR OF FOUR POUNDS OF ALFALFA HAY FED AT VARYING INTERVALS BEFORE MILKING.

The results in all three of the tables given above are more readily apparent when the data are plotted as in figure 1. The top curve in this figure shows the scores on all 12 cows in the trial. It is plotted from the values in the right-hand column in table 1. The middle curve in the figure repre-

sents the data on the Holsteins from table 2 and the bottom curve on the Jerseys from table 3.

#### IDENTIFYING THE ALFALFA FLAVOR

We have been impressed with the fact the alfalfa hay imparts a characteristic flavor. With even slight experience one is able to distinguish the flavor from the silage or pasture flavor. Though the flavor is pronounced it was confused with "cowy" when first encountered at the start of the trial. As now recognized after considerable experience the milk carrying the alfalfa flavor gives a slightly sweet sensation when first taken into the mouth. Then a slight bitter or acrid taste appears and it persists for a few minutes even after the mouth is rinsed with warm water. There is some analogy between this flavor and that obtained from tasting good alfalfa hay. The odor as well as the taste is pronounced.

#### COMPARISON OF ALFALFA HAY AND SILAGE

In a trial in which darso silage instead of alfalfa hay was studied results were secured that were quite unexpected. The darso silage in 12-pound quantities was consistently less offensive in producing off-flavors in the milk than was the alfalfa hay in 4-pound quantities.

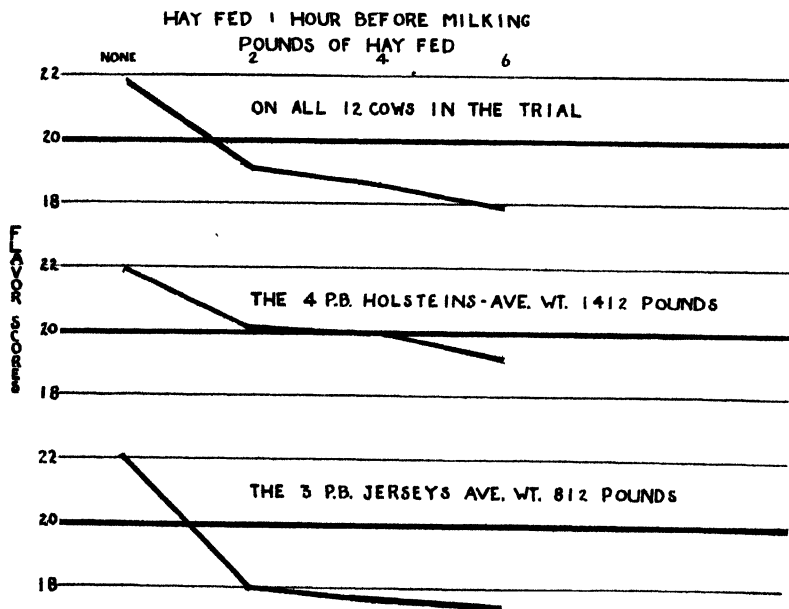


FIG. 2. EFFECT ON MILK FLAVOR OF VARYING AMOUNTS OF HAY FED ONE HOUR BEFORE MILKING.

The darso silage, while characteristic of this type of silage, was not of the quality that is found in good corn silage. It lacked the desired sharp odor of silage; it was also lighter and more fluffy. Possibly these reasons explain why it did not give as serious defects to the milk as did the hay.

It was of interest to observe that mangels in quantities of 20 to 35 pounds caused no injury to the milk flavor.

#### THE QUANTITY OF HAY FED

In this work some attention was devoted to the effects of different amounts of alfalfa hay on the milk flavor. Quantities consisting of two, four and six pounds were fed one hour before milking. Figure 2 gives the results secured in this phase of the trial.

From figure 2 it is observed that the 2-pound quantity of hay decreased the flavor score noticeably. The larger quantities occasioned further reduction in the scores but not in direct proportion to the amounts. Here, as in figure 1, it is observed that the Holsteins show far less effect than the Jerseys.

#### CONCLUSIONS

1. Alfalfa hay fed less than four hours before milking has a pronounced effect on milk flavor. This effect is observed even when the interval between feeding and milking is only one-half hour. The 2-hour interval causes the most serious flavor in the milk.

2. If the hay is fed as long as four hours before milking the flavor is entirely eliminated with some cows. With other cows it is so reduced as to be scarcely discernible.

3. The same precautions should be used in feeding alfalfa hay as are recommended for such feeds as silage; it should be fed after milking or at a sufficient interval before so that its possible effect on the milk flavor will have been eliminated.

4. The intensity of the alfalfa flavor in the milk increases as the amount of hay fed the cow is increased.

5. The effect of the hay on the milk flavor is considerably less serious with Holsteins than with Jerseys.

6. Aeration of the milk removes some of the flavor but does not entirely eliminate it. Cooling seems to be ineffective.

7. The effect of alfalfa hay is far more serious than that of darso silage.

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# THE PREPARATION AND NUTRITIVE VALUE OF A.I.V. SILAGE FOR DAIRY COWS\*

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## LITERATURE REVIEW

During the last few years a number of attempts have been made to perfect improved methods of ensiling fodder. For the most part these efforts have been directed toward the prevention of losses of nutrients, the prevention of the formation of objectionable substances in silage and toward the ensiling of crops which because of high protein content or other feature have not been customarily ensiled. Methods have been suggested by Hölken (1) and others for the application of heat from the outside or for the regulation of the amount of heat developing within the silage. Gorini (2) has obtained good results with this latter method. The object of heating is to produce a temperature favorable for the development of lactic acid bacteria but unfavorable for objectionable forms.

Considerable work has been directed toward overcoming the difficulties involved in ensiling legumes. In general protein decomposition is the greatest obstacle to such a practice, involving as it does, a loss of valuable nutrients and the production of compounds such as ammonia which are undesirable from the standpoint of odor and palatability. Schieblich (3), Shutt (4), and Wright and Shaw (5) have reported favorably on the practice of wilting legume crops before ensiling. Neal and Becker (6), Ragsdale and Turner (7), and Westover (8) were able to prepare good legume silage without wilting; but the loss of crude protein as reported by Neal and Becker was 34 per cent. Various substances have been added to legume crops during the process of ensiling in order to hasten lactic acid production and so to inhibit proteolytic enzymes of both plant and bacterial origin. Kuchler and Wachter (9), Shutt (4), and Schmidt (10) have tested the efficacy of these methods.

Rapid formation of lactic acid, although desirable from the standpoint of protein preservation, involves necessarily the destruction of a part of the carbohydrate content of the silage. In order to prevent this as well as to insure complete preservation of proteins Virtanen (11-17), has worked

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out a method of silage preservation entailing the addition of acids or acid compounds to the green fodder. Various mixtures of  $\text{HCl}$  and  $\text{H}_2\text{SO}_4$  may be used, and the mineral acids may be replaced by other compounds such as lactic acid,  $\text{NaHSO}_4$ ,  $\text{S}_2\text{O}_3\text{Cl}_4$ ,  $\text{SOCl}_2$ ,  $\text{SO}_2\text{Cl}_2$ , and  $\text{ClHSO}_3$ . The acid is sprinkled on the fodder in sufficient quantity to produce a pH of from 3.6 to 4.0. According to Virtanen, fodder with a pH of less than 3.0 is unsuitable for consumption, and if the pH is above 4.0 proteolysis and deamination take place with consequent loss of feeding value. It is claimed for this method that it checks plant cell respiration and bacterial activity, inhibits proteolysis, preserves soluble carbohydrates, carotene, and vitamin C, and prevents losses due to poor weather conditions at the time of harvesting the crop. Virtanen states that silage prepared according to this method contains from 0.52 to 0.80 per cent of lactic acid on the fresh basis and from 0.28 to 0.38 per cent acetic acid. When clover silage was preserved at a pH of 3.7, there was no increase in water soluble nitrogen or ammonia nitrogen; however, if the pH was 4.1, the ammonia nitrogen increased to 12 per cent of the total nitrogen, and the water soluble nitrogen increased from 26 to 44 per cent of the total nitrogen. There was no appreciable loss of carotene and only 50 per cent destruction of vitamin C, which, as is well-known, is completely destroyed in the process of making hay.

Legume silage prepared in this way, because of its relatively high protein content, has been substituted with good results for part or all of the concentrates of a winter ration. The possibility of thus eliminating high-priced concentrates is of considerable importance. According to Virtanen, cows receiving such a winter ration maintained their butterfat production at summer levels and the butter made from their milk simulated summer butter, having a higher carotene content and a higher iodine number than ordinary winter butter. The cows manifested no ill effects due to the acid, either from the standpoint of general condition or from the standpoint of the ash content of the bones and other organs. In order to compensate for the mineral acid added to the forage, chalk should always be included in the ration.

This method of preserving fodder has been named the A.I.V. method after Professor A. I. Virtanen, its sponsor and originator. It has found widespread use in Finland and Sweden and is protected by patents in those countries and in the United States, Canada, and Great Britain.

Sjöberg and Köhler (18) investigated the A.I.V. method of preserving cabbage leaves with  $\text{HCl}$ . The lowest pH attained was 3.8, and they found that at this acidity there was a very slight loss of carbohydrate and of protein, but no appreciable amounts of ammonia or organic acids were produced. A pH of 4.5 was sufficiently low to suppress completely the formation of butyric acid.

Other methods of preserving fodder by the addition of acid have been described by Gerlach (19) and Ruschmann (20), but neither of these has come into such general use as has Virtanen's method.

The experiments described herewith were undertaken for the purpose of investigating various aspects of the A.I.V. method, particularly with reference to the preservation of protein and carotene, the presence or absence of fermentative changes, the effect upon cows of feeding the silage as part of a winter ration, and the adaptability of the method to conditions of silage making in this country.

#### PART I. PREPARATION AND COMPOSITION OF SILAGE

##### *Filling of Silo*

Three lots of silage were put up, designated as Alfalfa I, Alfalfa II, and Soy Beans, and consisting respectively of 11.2 tons, 17.1 tons, and 9.7 tons. The amounts of acid to be added were determined according to the procedure described by Virtanen. Representative samples were taken from the field a day or two before filling the silo and chopped up. Each of three 200 gram samples was then ground in a mortar with about 100 cc. of distilled water. To the three samples were added respectively 6, 12, and 18 cc. of 2 N acid. The plant material and acid were thoroughly mixed in the mortar and then transferred with as little water as possible to glass jars in which the acidified plant material was pressed down by means of a bottle filled with water and allowed to stand for 24 hours. After this length of time each sample was thoroughly mixed in a large evaporating dish, part of the juice was pressed out, and the pH of the liquid was determined by either the quinhydrone or the glass electrode. If none of the three samples gave exactly the desired pH, the pH values were plotted against amounts of acid added; and from the curve, which is practically a straight line, the amount of acid necessary to produce a pH of 3.5 was determined. This amount was increased by 10 per cent to provide a margin for errors in sampling. As may be seen from table 1, the amount of acid required

TABLE 1  
*Quantity and cost of acid used per ton of silage*

ITEMS	ALFALFA 1	ALFALFA 2	SOY BEANS
2 N acid, HCl-H <sub>2</sub> SO <sub>4</sub> , l.	90	120	100
Commercial HCl, 18° B $\acute{e}$ , lbs.	30.5	48.2	40.2
“ H <sub>2</sub> SO <sub>4</sub> , 65° B $\acute{e}$ , lbs.	8.6	9.7	7.9
HCl			
H <sub>2</sub> SO <sub>4</sub> , mols	2.75	3.92	3.92
Cost of HCl, cts.*	48.8	77.2	64.3
“ “ H <sub>2</sub> SO <sub>4</sub> , cts.*	13.7	15.5	12.6
Total cost of acid, cts.*	62.5	92.7	76.9

\* Prices as quoted in *Oil, Paint and Drug Reporter*, July 30, 1934.

varied considerably, this variation depending upon the state of the crop when ensiled. The second lot of alfalfa was more mature than the first, which probably explains the higher acid requirement.

The addition of the acid was accomplished by various methods. In the case of the first lot of alfalfa, the acid was hoisted to the top of the silo in 20 liter bottles and siphoned out, or forced out with a small air pump, through a sprinkler onto the alfalfa which was run into the silo through a cutter in the usual way. In the case of the second lot of alfalfa, due to the proximity of the silo to an elevated driveway, it was possible to mix the acid at the top of the silo and siphon it into the silo. The alfalfa was not cut up and blown into the silo but was pitched in from the top. These conditions could not ordinarily be duplicated except by the use of a pit silo such as is employed in Finland. This lot of alfalfa and the soy beans were put up under the supervision of Mr. G. L. Rosenquist of the Valio Laboratories, Helsinki, Finland. The acid applied to the soy beans, which were cut up as was the first lot of alfalfa, was pumped to the top of the silo through a garden hose by means of an electrically-driven centrifugal pump. The stream of acid was delivered to the mouth of the blower pipe so that it was mixed thoroughly with the cut fodder emerging from the pipe. The proportions of hydrochloric and sulfuric acids were varied as indicated in table 1. From the standpoint of economy it is best to use as high a proportion of  $H_2SO_4$  as possible; but from the standpoint of feeding, it is not desirable to have too great a quantity of sulfates in the silage.

The most important factor in determining the cost, however, is the quantity of acid required. The lowest figure in table 1, 62.5 cents per ton, corresponds to an application of 90 liters. It is probable that in many cases, *e.g.*, with more succulent materials, the quantity of acid and hence the cost could be reduced. The cost would also vary with the quantity of acid purchased in a single order. The prices used in calculating the figures given in table 1 were those quoted for lots of 25 carboys or more, *viz.*, \$1.60 per 100 lbs. for both commercial muriatic acid and commercial sulfuric acid.

The first lot of alfalfa and the soy beans were covered with layers of corn silage about 2 feet thick. The second lot of alfalfa was covered as recommended by Virtanen. Double portions of acid were added to the last 1000 pounds of fodder, and to the last portion of acid was added about 100 cc. of a mustard oil emulsion in order to prevent mold growth. The surface of the silage was covered with tar paper on top of which was placed a 4 inch layer of moistened shavings and an 18 inch layer of soil.

The silo in which the second lot of alfalfa was ensiled was provided with a drain so that the liquid draining to the bottom could be removed, collected, and analyzed.

The fresh plant material and the silage were analyzed for dry matter, total nitrogen, total water soluble nitrogen, amino nitrogen, ammonia nitro-

gen and carotene. To a limited extent the bacterial population and the organic acid content of the silage were also investigated.

### *Composition of Silage*

The losses from the second lot of alfalfa silage by drainage of juice are set forth in table 2. It may readily be seen that the losses of dry matter

TABLE 2  
*Losses from alfalfa silage 2 by drainage of juice\**

	DRY MATTER	NITROGEN	2 N ACID
Silage	kg. 5030	kg. 153	liters 2075
Lost in drainage juice	89.2	1.40	237
Percentage loss	1.77	0.91	11.4

\* Total volume of drainage juice was 1,476 liters.

and of nitrogen in this way were negligible. The loss of acid, expressed in liters of 2 N acid, corresponded quite closely to the 10 per cent excess of acid added to allow for this loss. As might be expected, the first juice to drain out was the most acidic, having a pH of 1.37; whereas the last to drain out, 13 days after the filling of the silo, had a pH of 3.73.

Tables 3 and 4 show some of the changes occurring in plant tissue when preserved as A.I.V. silage over a period of 4½ to 5½ months. It was of course impossible to obtain a single sample representative of the contents of the silo as a whole and this fact together with the possibility of drainage downward of acid and of soluble nitrogen compounds probably accounts for the variable results obtained on different samples from the same silo. The high content of water soluble nitrogen would increase the possibility of downward movement of nitrogen. In this connection it should be mentioned that the low pH value of 2.1 recorded for the soy beans and the high figure for ammonia nitrogen recorded for the second lot of alfalfa were both obtained on samples taken from near the bottom of the silo. The pH values, except that mentioned above correspond fairly well with Virtanen's recommendations. The nitrogen analyses show marked increase in water soluble nitrogen and amino nitrogen, but no significant amounts of ammonia were found. Even in the above mentioned case, the second lot alfalfa sample, taken from near the bottom of the silo, contained only 0.1 per cent of ammonia nitrogen. The marked increases in water soluble nitrogen are not in accord with the results obtained by Virtanen who found, as stated above, that when the pH was 3.7 there was no increase in water soluble nitrogen. The pH was higher than this in the second lot of alfalfa, which fact would explain the difference in this case, but this explanation does not apply to the other two silages. The increases in water soluble nitrogen

TABLE 3  
*Analyses of fresh plant material and silage*

	PH	DRY MATTER	TOTAL NITROGEN DRY BASIS	WATER SOLUBLE NITROGEN, DRY BASIS	AMINO NITROGEN, DRY BASIS	AMMONIA NITROGEN, DRY BASIS	CAROTENE, DRY BASIS
		%	%	%	%	%	Micrograms per gm.
Alfalfa 1 Plant material		34.6	2.65	0.818	0.386	0.0289	129**
Silage	3.3	29.8	2.51	1.128	0.462	0.0288	139
Alfalfa 2 Plant material		32.3	3.04	0.446	0.190	0.0065	57**
Silage*	3.9-4.2	30.8 38.3	2.83 3.25	1.149 1.257	0.457 0.677	0.0359 0.1016	78 83
Soy Beans Plant material		27.2	2.57	0.514	0.254	0.0159	88**
Silage*	2.1-3.9	23.1 25.1	3.03 3.11	1.064 1.085	0.459 0.499	0.0177 0.0519	131 217

\* Analyses of 2-4 samples from various depths in silo.

\*\* These figures are low because of incomplete extraction.

TABLE 4  
*Nitrogen distribution in fresh plant material and silage*

	TOTAL NITROGEN	WATER SOLUBLE NITROGEN	AMINO NITROGEN	AMMONIA NITROGEN
	%	%	%	%
Alfalfa 1				
Plant material	100	30.9	14.6	1.09
Silage	100	44.9	18.4	1.15
Alfalfa 2				
Plant material	100	14.7	6.25	0.21
Silage	100	35.4-44.4	14.1-23.8	1.26-3.12
Soy Beans				
Plant material	100	20.0	9.88	0.62
Silage	100	34.9-35.1	14.7-16.5	0.58-1.67

TABLE 5  
*Data on fermentation in silage*

	ORGAN- ISMS PER GM. OF SILAGE	ORGANIC ACIDS, FRESH BASIS			
		Volatile, as acetic	Non-vola- tile as lactic	Lactic	Total, as lactic
	millions	%	%	%	%
Alfalfa 1 After 2 weeks	8				1.10
Alfalfa 2 After 5 months	1.2	0.70	1.72	0.946	2.76

and amino nitrogen are probably not of very serious consequence since increases in these forms of nitrogen do not necessarily mean decreased feeding value; whereas an increase in ammonia nitrogen detracts from both palatability and feeding value, since ammonia is unavailable to the animal.

Increases in the carotene content of the silage as compared with the fresh plant material were obtained consistently, and the increases were quite significant in most cases. These increases were very puzzling until after the experiment was completed—when it was found that treatment of fresh plant tissue with acid for 24 hours increased the carotene values markedly. It appears then that these apparent increases may be due merely to the action of the acid in increasing the extractability of the carotene.

The data on fermentation in the silage (table 5), though fragmentary, indicate that bacterial changes are by no means entirely suppressed. The bacterial count of 8 million organisms per gram obtained on the first lot of alfalfa two weeks after ensiling, while not an exceptionally high count, indi-

cates considerable bacterial growth. The later count made on a sample from the second lot of alfalfa 5 months after ensiling shows that a considerable number of microorganisms persisted even after this length of time. The amounts of volatile and non-volatile acids are approximately those found in ordinary silage. However, only the volatile and lactic acids should be attributed to fermentation process. The difference between non-volatile acid and lactic acid (0.67%) probably represents plant acids present in the original green alfalfa. The 0.946 per cent of lactic acid is somewhat in excess of the 0.52 to 0.80 per cent reported by Virtanen. Since the pH of the second lot of alfalfa silage ranged from 3.9 to 4.2, these results perhaps should not be compared with those of Sjöberg and Köhler who found that no appreciable quantities of organic acids were formed in cabbage preserved at a pH of 3.8. Factors other than acidity, as for instance the chemical and physical characteristics of the plant material, probably account for this great difference in organic acid content of silages differing only slightly in pH. The relatively high organic acid content of this lot of silage might well be expected on the basis of the rather large amount of bacterial activity indicated by the bacterial counts.

## PART II. FEEDING EXPERIMENT

### *Rations and Feeding of Animals*

Two lots of five dairy cows each were used in a double reversal feeding trial which extended over a six week fore-period, a seven week A.I.V. period, and a seven week after-period. During the control periods Lot I (three Holsteins and two Guernseys) was fed a grain mixture consisting of equal parts of corn and oats, plus alfalfa hay and corn silage, while Lot II (two Guernseys, two Jerseys and one Brown Swiss), received a grain mixture, 40 per cent corn, 25 per cent oats, 20 per cent gluten meal, and 15 per cent linseed meal, plus timothy hay and corn silage. A pound of grain was fed for every  $3\frac{1}{2}$  pounds of milk produced and 1 pound of hay and 3 pounds of corn silage per 100 pounds of live weight. In the A.I.V. silage period all cows received a 50:50 corn-oats mixture, half the former amount of alfalfa or timothy hay, no corn silage and A.I.V. alfalfa silage *ad libitum*. Approximately one per cent of iodized salt was added to the grain mixtures. In order to compensate partially for the high acid intake on the A.I.V. ration, 4 ounces of  $\text{CaCO}_3$  were fed daily in the grain mixture of each cow. Accurate records were kept of the amount of feed consumed, weight of cows, and milk and butterfat produced.

Lots I and II were not set up for the purpose of comparing one with the other but were conveniently divided in that manner because of the regular feeding schedule in use with the university herd. The cows in Lot I had been receiving alfalfa hay as their dry roughage prior to the beginning of the experiment, while the cows in Lot II had been getting timothy

hay. The cows were stall fed and had access to drinking water at all times.

At the beginning of the A.I.V. period the cows did not relish this higher acid taste, but, by increasing gradually the amount of A.I.V. silage fed, at the end of two weeks they were consuming from 30 to 50 pounds daily. On an average the cows consumed 50 per cent more A.I.V. silage than corn silage on the dry matter basis due to the fact that they were receiving only half as much hay. After the first two weeks of feeding none of the cows indicated an apparent dislike for this new type of silage. However, the feces of the cows on A.I.V. silage became very soft simulating the droppings of animals on pasture, a condition which was particularly noticeable in the group receiving timothy hay.

### *Effect on Milk Production*

Butterfat and milk production records indicated that the feeding of A.I.V. silage did not appear to have any unusual influence on milk production as the lactation curves from the two groups of cows appear to be approximately normal (Figure 1). However there were some irregularities

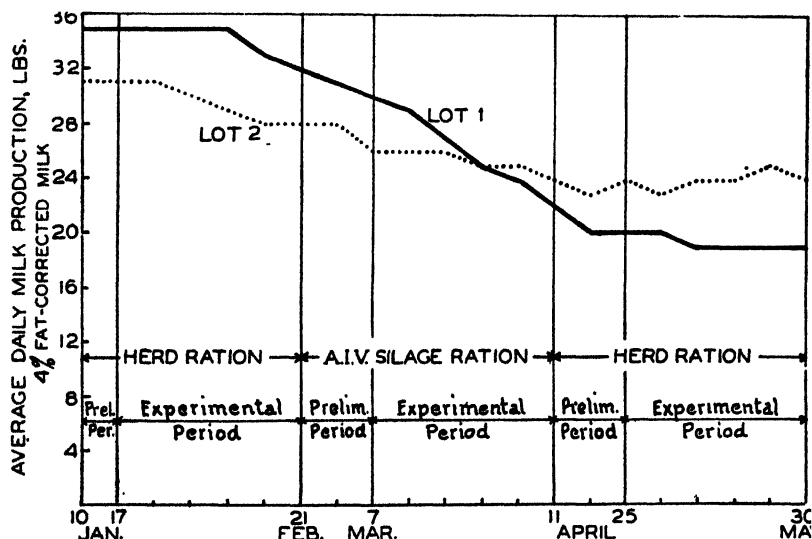


FIG. 1. EFFECT OF A.I.V. ALFALFA SILAGE ON MILK PRODUCTION

in the production curves during A.I.V. feeding which were probably due to the low intake of the silage during the adjustment period. A seven-week trial does not present enough data to be conclusive as to its effect on milk production. This can only be answered by experiment of longer duration, such as an entire lactation period.

*Effect on the Carotene and Vitamin A Control of Butterfat*

Individual butter samples were prepared from a composite 24 hour milk sample of cows of the same breed at definite intervals during the three periods. Both carotene and vitamin A content of the butter samples were determined spectroscopically by methods previously described (21, 22). These analyses indicated a definite increase in both carotene and vitamin A with the inclusion of A.I.V. silage in the ration (Figure 2). In most cases

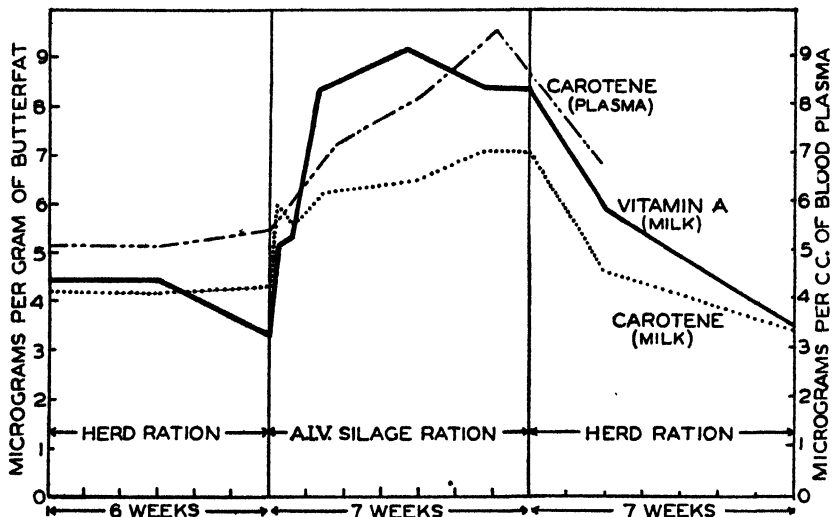


FIG. 2. INFLUENCE OF A.I.V. SILAGE ON THE CAROTENE AND VITAMIN A CONTENT OF MILK AND THE CAROTENE CONTENT OF BLOOD PLASMA (AVERAGE FROM TEN COWS OF VARIOUS BREEDS)

the maximum content of both of these components was reached in 4 weeks. This was a somewhat slower increase than we have found with cows on pasture (21), but it is probably due to the low intake of silage during the first two weeks of feeding. An average of the carotene for ten cows during the control period gave a value of 4.2 micrograms of carotene per gram of butterfat with an increase to 6.8 micrograms per gram during the A.I.V. period. Vitamin A analyses showed an average value of 4.0 micrograms per gram of butterfat for the control period and a definite rise to 9.0 micrograms per gram in the A.I.V. period. Both carotene and vitamin A indicated a definite drop within two weeks after the A.I.V. silage was discontinued, and were definitely at the control period level after seven weeks. The changes in carotene and vitamin A are shown more clearly by comparing the differences within the same breed. It was found in this study as well as in previous work (21) that the Holstein milk is high in vitamin A and low in carotene, Guernsey milk is low in vitamin A and high in caro-

tene, and milks from Jerseys and Brown Swiss occupy intermediate positions. Therefore when the results were averaged the breed differences in these components tended to neutralize the changes.

Although the feeding of A.I.V. silage gave a definite rise in both carotene and vitamin A, the values obtained were not so high as those obtained by a previous study with cows on pasture (21). A.I.V. silage gave a value of 9.5 micrograms per gram for the carotene of Guernsey butterfat, while the same cows on pasture yielded a butterfat with 17.0 micrograms of carotene per gram. The vitamin A of the Holstein butterfat was increased from 10 to 15 micrograms per gram by pasture feeding. The other breeds showed similar differences between A.I.V. and pasture feeding in both carotene and vitamin A (Table 6). These differences were no doubt due

TABLE 6  
*Effect of Winter ration, A.I.V. silage ration, and pasture on the carotene and vitamin A content of butterfat*  
(In micrograms per gram of butterfat)

BREED	CAROTENE			VITAMIN A		
	Winter ration 180 days	A I.V. alfalfa silage 49 days	Pasture 20 days*	Winter ration 180 days	A I.V. alfalfa silage 49 days	Pasture 20 days*
Guernsey	6.0	9.5	17.0	2.9	8.5	8.7
Holstein	3.5	5.4	6.6	4.0	10.0	15.0
Jersey	3.7	7.1	10.7	3.1	7.7	10.2
Brown Swiss	3.4	5.0	9.8	5.8	9.7	12.6
Ave.	4.2	6.8	11.0	4.0	9.0	11.6

\* These results were taken from analyses of butters from various breeds within the herd prior to this experiment.

in part to a lower consumption of A.I.V. silage than pasture, thus resulting in a lower carotene intake. The lower consumption of A.I.V. silage as compared to pasture may be partly attributed to the fact that the cows were receiving hay with A.I.V. silage, while the pasture cows received no dry roughage.

Studies on the carotene content of the blood plasma paralleled the increases in the butter (Figure 2). The average carotene content of the plasma on a normal ration was 5.2 micrograms per cc. with a definite increase to 9.6 micrograms per cc. on the A.I.V. ration (Figure 2).

Balance studies on the total carotene intake in the feed and the total carotene and vitamin A output in the milk indicated that approximately doubling the carotene consumption due to A.I.V. feeding also doubled the total vitamin A content of the milk (Table 7). Expressed in terms of total

TABLE 7  
*Carotene balance of cows on herd and A.I.V. ration*  
*(Average carotene intake and output per animal)*

	LOT I		LOT II	
	Herd ration	A.I.V. ration	Herd ration	A.I.V. ration
Carotene in 10 lbs. grain, mg.*	10	9	18	8
Carotene in roughage, mg.	347	552	180	502
Total carotene fed daily, mg.†	357	561	298	510
Total vitamin A output daily‡	6,644	11,389	5,894	10,785

\* Carotene content per pound was as follows: grain mixture of Lot I 0.091 mg.; grain mixture of Lot II 1.8 mg.; alfalfa hay 11.5 mg.; timothy hay 2.1 mg.; corn silage 5.9 mg.; and A.I.V. alfalfa silage 13 mg.

† 1 mg. of carotene is equivalent to 1000 International rat units of vitamin A.

‡ International rat units of vitamin A.

international units of vitamin A produced on an average by each cow, our figures showed that during the control period the cows secreted in the milk on an average 6,269 units of vitamin A per day and on the A.I.V. ration the figure was raised to 11,087 units. Calculations of the intake and output of carotene in the same units showed that the animals received an enormous excess of carotene over the amount secreted. On both rations only 2 per cent of the ingested carotene of the feed appeared in the butterfat. Ordinarily an increase in the carotene ingested decreases the percentage absorbed (21), but with the addition of A.I.V. silage to the ration this did not appear to be the case. There is evidently a tremendous amount of the ingested carotene unabsorbed, or some of it is destroyed by certain acids and micro-organisms (24, 25). Furthermore all the carotene absorbed is not secreted in the butter but may be either stored in the tissues, utilized directly by the tissues, or converted into vitamin A. A study of the actual percentage of ingested carotene utilized by the cow would no doubt yield interesting results.

#### *Nutritive Value of A.I.V. Milk*

In connection with a larger program on the nutritive value of milk produced at different seasons of the year (23), the nutritive value of A.I.V. milk was determined by feeding experiments with rats. The A.I.V. milk, fortified with Fe, Cu, and Mn, was fed *ad libitum* for six weeks to 6 male rats having an initial weight of 50 grams each. The rats receiving A.I.V. milk made an average gain in six weeks of 144 grams, while controls fed winter milk, fortified with the same elements, gained only 102 grams. This increase in growth was probably not due to the greater supply of vitamin A in the A.I.V. milk (37 units per day) since the controls received 17 rat units

per day, an intake which is many times the amount needed to meet the vitamin A requirement. The reasons for the superior growth of rats on the A.I.V. milk cannot be explained at the present time. Further research is in progress to throw some light on this question.

*Effect on the Composition of the Blood and Urine*

Five blood samples were analyzed at definite intervals during the course of the experiment in order to determine the effect of the high acid intake on their composition. The blood was collected under paraffin oil using sodium oxalate as an anti-coagulant, centrifuged immediately and the pH determined at 25° C. with a quinhydrone electrode in a closed chamber (26). The alkaline reserve was determined with a Van Slyke manometric blood gas apparatus (27). The alkaline reserve of the blood was lowered on an average from 66.3 cc. per cent for the control period to 56.7 cc. per cent

TABLE 8  
*Effects of feeding A.I.V. silage on composition of blood and urine*

BLOOD PLASMA	FOR- PERIOD	A.I.V. PERIOD			AFTER- PERIOD
	3 week average	2nd week	4th week	6th week	2nd week
Alkaline reserve, cc. %					
Lot I—Alfalfa-fed cows	63.8	61.2	61.7	59.5	71.0
Lot II—Timothy-fed cows	63.8	53.9	53.1	53.8	66.1
pH					
Lot I	7.45	7.40	7.42	7.43	7.48
Lot II	7.45	7.37	7.40	7.40	7.45
URINE	2 days before	3rd week		6th week	2nd week
Fixed CO <sub>2</sub> , cc. %					
Lot I	519.4	2.6			405.8
Lot II	31.8	4.0			79.2
pH					
Lot I	8.08	5.76		6.13	8.01
Lot II	6.97	6.08		6.15	7.20
NH <sub>3</sub> -N, gm./100 cc.					
Lot I	.0006	.081		.120	.009
Lot II	.019	.133		.131	.034
Total-N, gm./100 cc.					
Lot I	.75	.84		.89	1.47
Lot II	1.68	1.12		1.16	1.85
Percentage of total N as NH <sub>3</sub> N					
Lot I	.08	9.6		13.5	.61
Lot II	1.13	11.9		11.3	1.84

for the A.I.V. period (Table 8). These reductions due to A.I.V. feeding are not great enough to bring about a condition of acidosis. The slight

lowering of the pH of the blood is insignificant (Table 8). There was a prompt return of the alkaline reserve and pH to the former levels within two weeks after the feeding of A.I.V. silage was discontinued.

Four urine samples from each cow were analyzed. pH was measured with the quinhydrone electrode, fixed  $\text{CO}_2$  with the Van Slyke manometric blood gas apparatus (27), ammonia nitrogen by the Folin aeration method as modified by Steel (28), and total nitrogen by the Kjeldahl method. The fixed  $\text{CO}_2$  content of the urine indicated a drastic reduction from the control period to the A.I.V. period, the average being 276 cc. per cent for the former and 3 cc. per cent for the latter. The pH was lowered on an average from 7.5 to 5.9 and the ratio of the ammonia nitrogen to the total nitrogen was increased from 0.6 to 11.0 per cent (Table 8). The values for fixed  $\text{CO}_2$ , pH, and ammonia returned to normal after the cows were on the regular ration two weeks. These data show that more than an average quantity of acid was consumed by the cows and in part was neutralized by means which apparently prevented any deleterious effects on the health of the animals. However, it must be kept in mind that our observations extend over only a relatively short feeding period and it cannot be assumed that these results are sufficient to allow us to draw definite conclusions as to the ultimate effect of continuous feeding of A.I.V. silage.

#### SUMMARY

The preparation by the A.I.V. method of two lots of alfalfa and one of soy bean silage, consisting respectively of 10, 17, and 11 tons, is described.

The data show that the loss of nitrogen and of dry matter in the drainage juice amounted to 0.9 and 1.8 per cent respectively.

There were marked changes in the distribution of nitrogen in the silage. Based on the total nitrogen, the water soluble nitrogen increased in the two lots of alfalfa silage from 31 to 45 per cent and from 15 to 40 per cent respectively, and from 20 to 35 per cent in the soy bean silage. Increases in amino nitrogen paralleled those of the water soluble nitrogen. Ammonia nitrogen also increased, but the absolute quantity was small.

There was apparently no loss of carotene.

Counts of the number of bacteria and determination of volatile acids and lactic acid showed that some fermentation took place in spite of the low pH of the silage.

A double reversal feeding trial was conducted consisting of a six week fore-period, a seven week A.I.V. silage period, and a seven week after-period with ten dairy cows of various breeds in two groups of five each.

No unusual changes were noted in milk production due to the feeding of A.I.V. silage.

Spectroscopic analyses of the butterfat for carotene and vitamin A indicated a definite increase in these components with silage feeding. During

the control period an average value of 4.2 micrograms of carotene per gram of butterfat was obtained and in the A.I.V. period the figure rose to 6.8. Vitamin A analyses showed an average value of 4.0 micrograms per gram of butterfat for the control period and a marked rise to 9.0 in the A.I.V. period. However, the values for carotene and vitamin A during the A.I.V. feeding were not as high as those previously found for pasture feeding. Increases in the carotene content of the blood plasma paralleled those in the butterfat. Balance studies on the carotene intake in the feed and output in the milk showed a corresponding increase in both of these factors with the inclusion of A.I.V. silage in the ration.

Blood and urine analyses indicated that the high acid intake of the cows was neutralized by means which prevented any noticeable deleterious effects on the animals.

### ADDENDUM

After this manuscript was prepared the paper by Watson, *et al.* (Biochem. J. 28, 1076, 1934) giving the effect of feeding grass ensiled by the A.I.V. method on the carotene and vitamin A content of butter appeared. These authors report a marked increase in both carotene and vitamin A as a result of feeding this fodder.

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# American Dairy Science Association Announcements

## PROBLEMS IN TEACHING AT JUNE MEETING

President C. L. Roadhouse has indicated that the time will be ripe next June for another special session for the members of the Association who are interested in a discussion of teaching problems and methods. It is urged that the Heads of Departments of dairying and dairy husbandry in the various colleges who have inaugurated new curricula or innovations or modifications of such teaching practices arrange to have these presented and discussed at the June meeting of the Association. Inasmuch as there is no official Section of Dairy Instruction with duly elected officers, the Chairman of the Program Committee will be glad to arrange for temporary officers and the Program Committee will arrange a special program for such a temporary section if sufficient titles are sent in.

L. S. PALMER, *Chairman Program Committee*

## REPORT OF COMMITTEE ON EVALUATION OF PROVEN SIRE

The correct evaluation of a sire's transmitting ability requires a correct record of what his daughters have produced under standard conditions and somewhat less important, of what the dams of those daughters have produced under equivalent conditions.

The committee feels that more research on the subject and more experience in the actual use of these indexes will be required to justify official choice of any index. For this reason the committee is unwilling to recommend any specific index as correct and best under all conditions.

While practical experience in the use of these indexes is being increased, it is important that the fundamental data on which they rest should be presented for practical use in standardized averages such that those interested in the use of an index could easily compute any of the more promising indexes he may choose.

The committee, therefore, recommends to the Breeds Relations Committee that they ask the Breeds Association to publish the following information for all bulls with five or more tested daughters out of tested dams or with eight or more tested daughters even though less than five of these are out of tested dams:

1. The total number of registered daughters and the number of registered daughters more than four years of age.

2. The average production of all tested daughters. (By "production" is meant yield of milk, per cent fat, and total fat, corrected to a standard age and to a standard classification for number of times milked and to a standard length of lactation, preferably 305 days.)

3. The average production of all daughters out of tested dams.

4. The average production of those dams.

5. The number of daughters which exceed their dams.

6. The range or other measure of variation in the daughters' production.

Committee: F. W. ATKESON, Idaho; J. L. LUSH, Iowa; C. W. TURNER, Missouri; R. R. GRAVES, U. S. D. A.; H. O. HENDERSON, W. Virginia, Chairman.

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INCORPORATED IN THE DISTRICT OF COLUMBIA

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The American Dairy Science Association was organized to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

Membership shall consist of two kinds: (1) active, (2) associate.

The qualifications for membership in the two classes are as follows: (a) Any person is eligible to active membership who is formally announced by an Agricultural College, or Experiment Station, or by the Bureau of Dairying of the United States Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or (b) anyone filling a position of responsibility connected with the dairy industry and who has had a college or University training in technical science, or anyone filling a responsible position in the industry of a professional character requiring a technical knowledge of dairying of a high order.

Any person is eligible to associate membership who is regularly enrolled in a collegiate course in a college of Agriculture and who is specializing in dairying. Associate membership is attained by election to membership in a local chapter of the American Dairy Science Association.

The dues are \$5.00 a year for active membership. Correspondence regarding membership and dues should be addressed to R. R. Graves, United States Department of Agriculture, Washington, D. C.

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## COLOR DEVELOPMENT IN LACTOSE SOLUTIONS DURING HEATING WITH SPECIAL REFERENCE TO THE COLOR OF EVAPORATED MILK

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Washington, D. C.*

In the manufacture of those dairy products which are processed at high temperatures or which contain a large quantity of reducing sugar, an undesirable brown color develops. This color is especially noticeable in the manufacture of milk sugar, evaporated milk and sweetened condensed milk.

It is a matter of general experience in lactose manufacture that during the heating of the neutralized whey which contains considerable quantities of protein, a dark brown color develops which is greatly intensified as the reaction of the solution is made more alkaline.

In the case of evaporated milk, which contains approximately 10 per cent lactose and 6.7 per cent protein and is sterilized at 115° C. for fifteen minutes, a characteristic brown color develops during heating. Since the composition and processing of this product must be controlled within narrow limits, the possibility of avoiding a darkening in color appears slight. The brown color of evaporated milk is held by the casein after coagulation and cannot be washed from this protein nor removed from it by dissolving the coagulated and washed casein and re-precipitating it in acid solution.

Sweetened condensed milk, not being subject to extremely high processing temperatures, does not suffer excessive darkening in color during manufacture, but undergoes a marked color change in storage, especially at high temperatures (5).

There are many references in the literature dealing with the browning of sugar solutions during heating. If a pure sugar is heated dry or in an alkaline medium, caramel is formed, the reaction apparently being one of progressive dehydration and polymerization. If a reducing sugar is heated in alkaline or slightly acid solution in the presence of amino acids, a brown color develops, its intensity being chiefly dependent upon temperature, reaction of the medium and the amino acid and sugar concentration.

A reaction occurs at ordinary temperatures between reducing sugars and

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amino acids. The optical rotation of glucose shows a decrease upon the addition of glycine to the sugar solution (14).

The product formed when glucose is heated with an amino acid is complex in nature and has been shown to contain carbon, hydrogen, oxygen and nitrogen (10), (1).

There is evidence, especially in the case of cane sugar, that catalysts play an important rôle in the polymerization of sugars during heating. Metals, especially iron, have been found to catalyze the coloring of alkaline sucrose solutions at high temperatures, (3), (9), (11), (12), (13).

Caramel was prepared by Beal and Bowey (2) from glucose, using ammonium chloride or ammonium sulfate with hydrochloric acid. These salts catalyzed the reaction and did not seem to enter into the composition of the caramel.

Orla-Jensen and Plattner (7) first showed that the color which develops in heated milk could be attributed to the presence of both lactose and casein. Wright (17) considered the color to be due to a caramelization of the lactose which was catalyzed by calcium caseinate and that the casein in adsorbing the pigment remained unchanged.

Ramsey, Tracy and Ruehe (8) have reported some interesting experiments upon color development in skim milk subjected to various treatments. They consider the color to be due to a sugar-amino acid condensation, the speed of the reaction depending upon temperature, hydrogen-ion concentration, and the kind of sugar used.

Color changes caused by certain modifications in the manufacturing process of evaporated milk have been studied by Webb and Holm (15). They found that an increase in heat, whether encountered during forewarming, sterilization or storage, produced an increase in color, the change being of a catalytic nature.

#### EXPERIMENTAL

The work included in this report covers a series of experiments carried out on lactose solutions held during heat treatment at known pH values by means of suitable buffers. The color of the heated solutions was measured by comparison with color standards of known values.

Lactose solutions of the required concentration were made up from special lactose of a high degree of purity. Buffer solutions and solutions of amino acids and ammonium salts were prepared to give the desired molal concentration when mixed with a given quantity of lactose solution. All figures expressing concentration of lactose, buffers or salts in the different solutions are given as the concentration of the specified substance in the particular solution under consideration. The concentration of solutions from which the final mixtures were made have been omitted.

A total of 7 cc. of each mixture to be heated was measured into a pyrex

test tube, the upper part of which had previously been drawn out to facilitate sealing the tube. The tubes were sealed and heated in a glycerine bath held at  $120^{\circ}\text{C}$ . for different lengths of time. After rapid cooling, the color of the lactose solutions was measured by comparison with sealed tubes of standardized colors in a constant north light, using a special test tube rack with a white background. These comparisons were made by eye and it was found possible to check the measurements to  $\frac{1}{3}$  to  $\frac{1}{2}$  of the difference between any two standards. Therefore differences between standards are expressed as  $\frac{1}{3}$ ,  $\frac{1}{2}$  or  $\frac{2}{3}$  as the case might be.

TABLE I

*The composition of the color standards used for measuring the color of heated lactose solutions*

COLOR STANDARD NUMBER	COMPOSITION OF COLOR STANDARD EACH MADE UP TO 7 CC. WITH DISTILLED WATER
1	0.3 cc. of CPR-PR solution <sup>1</sup>
2	0.6 " " " " "
3	1.0 " " " " "
4	1.5 " " " " "
5	2.0 " " " " "
6	3.0 " " " " "
7	0.2 cc. Thymol Blue (0.04%) solution
8	0.3 " " " " "
9	0.45 " " " " "
10	0.7 " " " " "
11	1.1 " " " " "
12	1.5 " " " " "
13	2.0 " " " " "
14	7.0 cc. Molal $\text{FeCl}_3$
15	7.0 " $1\frac{1}{2}$ " "
16	7.0 " 2 " "

<sup>1</sup> Solution consisted in 3.8 cc. chlor phenol red concentration 0.04%, 0.6 cc. of phenol red, concentration 0.04%, and 10 cc. of HCl made up to 100 cc. with distilled water.

The colors developed in the lactose solutions during heating and storage were compared with color standards prepared according to table 1. These colors ranged from an almost water-clear solution (#1) to the dark reddish brown of 2 molal  $\text{FeCl}_3$  solution (#16). Reproduction of these colors is possible by following table 1. If difficulty is encountered with the thymol blue standards a very slight shift in the pH of the distilled water solutions will place them in their proper positions. The fact should be noted that different samples of  $\text{FeCl}_3$  may give slightly different shades of brown, depending upon the extent to which  $\text{Fe}(\text{OH})_3$  has formed in the sample used.

To be certain that the differences between standards represented equal differences in color it seemed desirable to check the standards more accurately than it was possible to do by eye. Therefore measurements expressed

in numerical terms according to the Munsell system (6) (15) were secured. The measurements were made upon a duplicate set of standards, the originals having already been sealed in test tubes which were not of appropriate size to measure in the colorimeter. Measurements were made on one-inch diameter areas of liquid placed in culture flasks of 1½ inches diameter backed by N 9.4 paper. To reduce the chroma in matching samples and color discs it was necessary to place a narrow strip of white across the sample which would make the corrected chroma approximately 10 per cent stronger than the values indicate.

The data obtained are shown in Fig. 1. It will be noted that, except

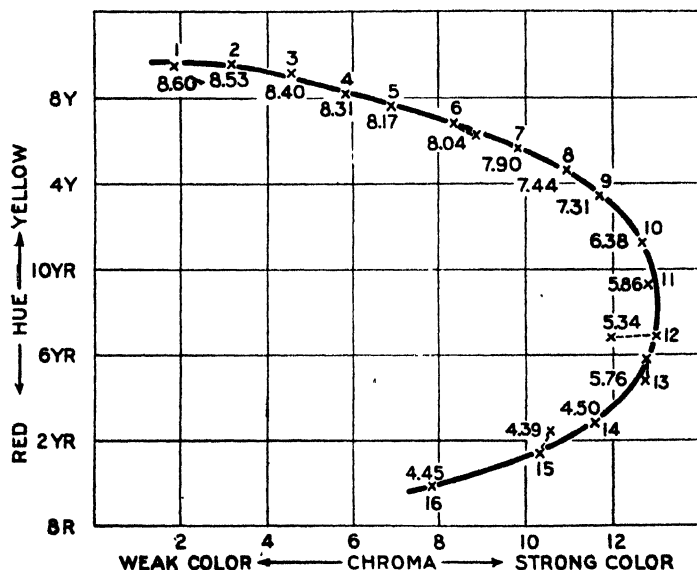


FIG. 1. NUMERICAL MEASUREMENTS OF THE 16 COLOR STANDARDS ACCORDING TO THE MUNSELL SYSTEM. THE FIGURES WITHIN THE CURVE REFER TO THE THIRD COLOR ATTRIBUTE, BRILLIANCE, AND THE GREATER VALUES REFER TO THE LIGHTER COLORS.

for small variations, the spacing of the standards along the curve is quite uniform, indicating the color difference between each to be approximately the same. The positions of standards 6, 12, 13, and 15 have been shifted to the curve from their measured positions because comparison by eye of the original sealed standards to the measured duplicates showed these positions to be more nearly those occupied by the sealed standards. In the present work the sixteen standards have been considered as being equally spaced over the range of color which they include.

The hydrogen-ion concentration of a lactose solution is of major importance in controlling color development during heating. This is especially true where the reaction is above pH 6.0; and when pH 7.0 is reached,

it requires very little heating to produce a deep caramel color. It was therefore deemed advisable to determine the pH of the solutions both before and after heating. These figures, determined potentiometrically by means of a quinhydrone electrode, are given in the accompanying tables and charts.

The effect of differences in pH can be noted in several of the following tables. The data given in table 2 show clearly the marked darkening in the color of 4 per cent heated lactose solutions as the pH is increased.

To counteract the acidity which develops in lactose solutions during heating and eliminate a variable in the experimental work, an efficient buffer is necessary. Several buffers were tried, the color and pH of each solution being measured after completion of the heating period.

TABLE 2

*The relationship in color development of 4% lactose solutions heated to 120° C. for 15 minutes, when buffered by sodium phosphate and by sodium maleate, with and without the addition of ammonium chloride*

B U F F E R	No $\text{NH}_4\text{Cl}$			$\text{NH}_4\text{Cl}$ CONCENTRATION = M/13		
	REACTION		COLOR	REACTION		COLOR
	Before heating	After heating		Before heating	After heating	
	pH	pH		pH	pH	
Sodium Phosphate buffer M/4.5	5.67	5.66	3- $\frac{1}{3}$	5.63	5.55	9- $\frac{1}{3}$
	5.90	5.84	4- $\frac{2}{3}$	5.87	5.76	12
	6.04	5.97	5- $\frac{2}{3}$	6.01	5.91	13
	6.27	6.19	7	6.24	6.12	14
	6.47	6.32	8- $\frac{1}{3}$	6.45	6.27	15
	6.66	6.45	9	6.63	6.41	16
	6.83	6.55	10	6.80	6.55	(17)
Sodium Maleate buffer M/1.5	5.47	5.47	1- $\frac{1}{3}$	5.42	5.40	7
	5.93	5.92	4- $\frac{1}{3}$	5.90	5.90	10- $\frac{1}{3}$
	6.23	6.22	6	6.20	6.15	13
	6.47	6.36	8	6.43	6.37	13- $\frac{2}{3}$
	6.54	6.38	8	6.51	6.40	14
	6.71	6.44	9	6.71	6.46	14- $\frac{1}{3}$

Some of the data obtained are plotted in Fig. 2. The figures on the ordinate are the product of the time of sterilization in minutes and the color intensity as expressed by the number of the color standard which the sample matched. The figures for evaporated milk are taken from the data of Webb and Holm (15) where different color standards were used.

Citrate and acetate were not satisfactory but phosphate and maleate buffers were very effective in maintaining a reaction comparable to that encountered in milk. Curves 3 and 4, representing M/2 maleate and M/2 phosphate solutions in 5 per cent lactose show strong buffering at the reaction of evaporated milk. If the lactose concentration is increased to 10 per cent as in curve 6, even molal phosphate is not sufficient to hold the

reaction constant. Much of the work reported below was carried out using  $M/2$  phosphate and  $M/2$  maleate solutions to buffer 5 per cent lactose.

The phosphate buffers were made up to the required pH by using appropriate proportions of  $\text{Na H}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$ . Maleate buffers were made up by adding concentrated  $\text{NaOH}$  to solutions of maleic acid of the specified concentration. Sufficient alkali was added to the maleic acid to give a solution of the desired pH. For maleic acid  $\text{pK}_1 = 1.93$  and  $\text{pK}_2 = 6.58$ , (4), the buffering of the second hydrogen being the one of interest in this work.

There has been considerable uncertainty in dealing with the changes occurring in milk during heating as to whether the phosphates present in normal milk act in a catalytic capacity. Whittier and Benton (16) in their work on the formation of acid in milk by heating concluded in part, "Experiments on lactose solutions containing radicals similar in their buffer action to those in milk, but chemically different, would settle the question

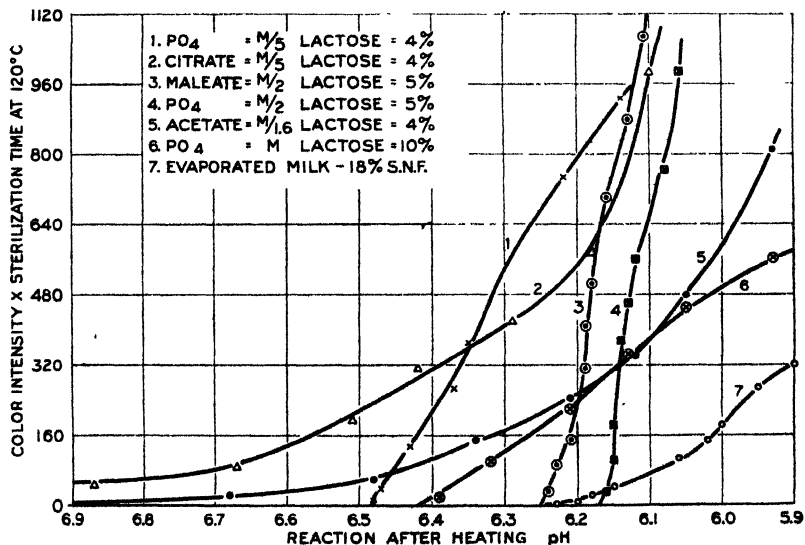


FIG. 2. COMPARISON OF THE CHANGE IN HYDROGEN-ION CONCENTRATION DURING HEATING WHICH OCCURS IN LACTOSE SOLUTIONS CONTAINING VARIOUS BUFFERS.

of a specific phosphate effect on reactions involving lactose. . . . Until it is shown that buffering acid radicals other than those present in milk but having practically the same dissociation constants, do not exert the same physical and chemical effects on the equilibria, we feel that any claims for specificity of action of phosphates in milk are unproved."

It appeared that a comparison of the color developed in lactose solutions with phosphate and maleate buffers would determine whether the phosphate itself was responsible for a catalytic influence when milk prod-

ucts are heated. Accordingly the data plotted in Fig. 3 were obtained comparing color development in these two solutions for different periods of heating. The curve for the phosphate solution shows the color of these samples to have been considerably darker than those containing maleate buffer.

Additional data were obtained to confirm the findings of Fig. 3. This

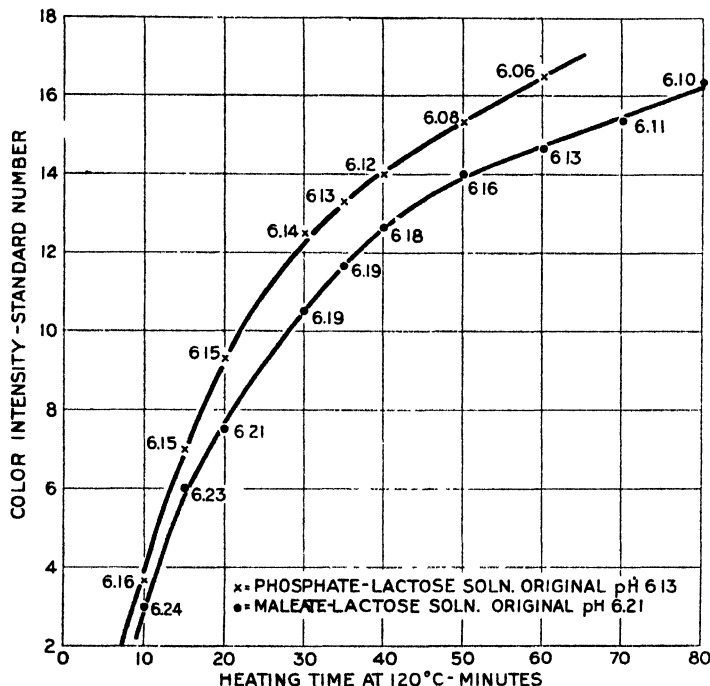


FIG. 3. COMPARING THE COLOR DEVELOPED DURING THE HEATING OF 5% LACTOSE SOLUTIONS IN M/2 PHOSPHATE AND M/2 MALEATE BUFFERS. THE FIGURES REFER TO THE pH AFTER HEATING. CONCENTRATION OF  $\text{NH}_4\text{Cl}$  CONSTANT AT M/23.

is reproduced in table 2 and again it is clearly indicated that phosphate exerts an effect favorable to increased color development during the heating of lactose solutions.

The amount of color formation promoted by phosphate-ions is dependent upon their concentration in the solution as shown by the data plotted in Fig. 4. The tubes containing the higher concentrations of phosphate showed much more color than those tubes having fewer phosphate-ions. The data was secured with and without  $\text{NH}_4\text{Cl}$  in the tubes.

Maleic acid is known to act as an anti-oxidant for fats and oils. A similar action in lactose solutions might produce an unduly light color and hence render a comparison between phosphate and maleate buffers of little

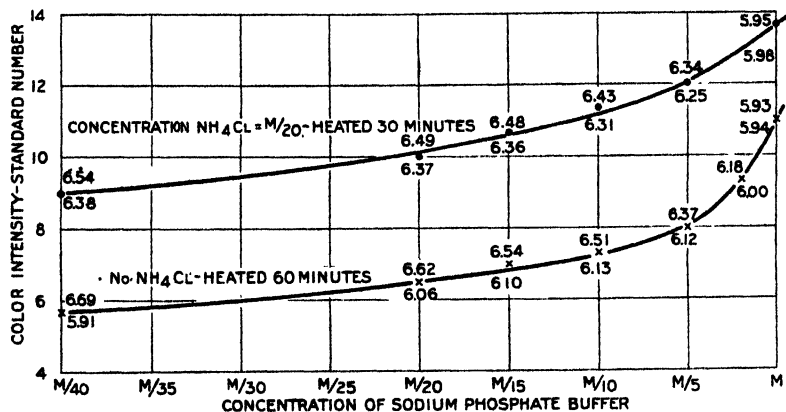


FIG. 4. THE INTENSITY OF COLOR DEVELOPED BY HEATING 5% LACTOSE SOLUTIONS AT 120° C. WITH AND WITHOUT NH<sub>4</sub>CL, USING PHOSPHATE BUFFERS OF DIFFERENT CONCENTRATIONS. THE UPPER FIGURES REFER TO THE pH OF EACH SOLUTION BEFORE HEATING, THE LOWER TO THE pH AFTER HEATING.

value. It did not seem probable however that sodium maleate would behave as an anti-oxidant in a non-fatty system such as a lactose solution. To be certain that the maleate buffer was not acting as an anti-oxidant in preventing color development and that mere traces of phosphate did not noticeably catalyze the reaction, the experiment reported in table 3 was carried out. Small quantities of either phosphate or maleate buffer in the presence of

TABLE 3

*The effect upon the color of 5% lactose solutions of the presence of a small quantity of phosphate when maleate buffer is used during heating and conversely when phosphate is the chief buffer*

Solutions heated to 120° C. for 30 minutes

PORTIONS OF M/2 BUFFER SOLUTIONS PER G CC. TOTAL SOLUTION. NH <sub>4</sub> CL CONSTANT AT M/20		REACTION		COLOR
MALEATE	PHOSPHATE	Before heating	After heating	
cc.	cc.	pH	pH	
6.0		6.22	6.20	10- $\frac{2}{3}$
5.9	0.1			10- $\frac{2}{3}$
5.5	0.5			10- $\frac{2}{3}$
1.0	5.0	6.15	6.11	12- $\frac{2}{3}$
0.5	5.5	6.12	6.10	12- $\frac{2}{3}$
0.1	5.9	6.12	6.12	12- $\frac{2}{3}$
	6.0	6.13	6.12	12- $\frac{2}{3}$

the other did not appreciably change the color of the solutions during heating.

The effect of lactose concentration upon color development was investigated using a maleate buffer and without the addition of an ammonium compound. The reaction was adjusted to simulate that encountered when milk is heated under like conditions. The results obtained are reproduced in table 4. It will be noted that a progressive increase in color follows the increases in lactose concentration.

TABLE 4

*The relationship between lactose concentration and color development during heating.  
Maleate buffer concentration =  $M/2$ .*

LACTOSE CONCENTRATION	REACTION		COLOR
	Before heating	After heating	After heating 120° C. for 30 minutes
<i>Per cent</i>	<i>pH</i>	<i>pH</i>	
2	6.55	6.51	5- $\frac{2}{4}$
4	6.56	6.44	7
6	6.56	6.41	7- $\frac{2}{4}$
8	6.54	6.37	8
10	6.56	6.34	8- $\frac{1}{4}$
12	6.55	6.32	8- $\frac{2}{4}$
15	6.55	6.26	9

A quantitative measurement of color development in buffered lactose solutions wherein the pH was held constant during heating was conducted, using different amino acids and ammonium compounds. The concentrations of these compounds were so adjusted that each tube of buffered lactose solution contained a concentration of ammonium or amino groups equal to  $M/70$ . In cases where this quantity of nitrogen compound produced little or no increase in color above the control, greater amounts were added. The results obtained are given in table 5. It will be seen that cystine, asparagine, the amides, and urea do not produce as much color as do the other compounds for a given quantity of  $NH_2$ . These results can perhaps be attributed to the extent to which these compounds enter into the reaction. The results of table 5 indicate that the darkening of the color of milk during heating is dependent upon the number of reactive amino groups which are present.

Different metals were added to lactose solutions before heating to study their effect upon color development. The data obtained in three different experiments are reproduced in table 6. Copper and iron caused a slight increase in color while tin showed a distinct tendency to lessen the color in-

TABLE 5

*The effect of nitrogen-containing compounds upon color development in 5% lactose solutions buffered by M/2 sodium phosphate*

CONCENTRATION OF COMPOUND IN LACTOSE SOLUTION		REACTION		COLOR AFTER HEATING TO 120° C -30 MIN.
		Before heating	After heating	
Check—nothing added		pH	pH	7
Glycine	M/70	6.17	6.16	9 $\frac{1}{3}$
Leucine	M/70	6.15	6.13	9
Cystine	M/70	6.17	6.14	7 $\frac{1}{3}$
Tyrosine	M/70	6.16	6.19	9 $\frac{1}{3}$
Asparagine	M/140	6.16	6.15	8
“	M/35	6.17	6.16	11 $\frac{1}{3}$
Tryptophane	M/140	6.17	6.16	9
Acetamide	M/70	6.15	6.13	7
“	M/14	6.16	6.14	7
“	M/1.75	6.23	6.37	7 $\frac{2}{3}$
Formamide	M/70			7 $\frac{2}{3}$
“	M/17.5	6.17	6.14	9
Urea	M/140			7 $\frac{1}{3}$
“	M/3.5	6.19	6.75	15 $\frac{2}{3}$
(NH <sub>4</sub> ) <sub>2</sub> C <sub>2</sub> H <sub>3</sub> O <sub>7</sub>	M/210			9
(NH <sub>4</sub> ) <sub>2</sub> C <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	M/140	6.16	6.13	9 $\frac{1}{3}$
(NH <sub>4</sub> )Cl	M/70	6.16	6.12	9 $\frac{1}{3}$

tensity. These results are of special interest in the case of evaporated milk manufacture since this product is evaporated in copper vacuum pans but is sterilized and held in tin cans. The effect of metals upon color development during the sterilization of evaporated milk can probably be considered unimportant, with the inhibitive effect of the tin can counteracting the darkening influence of copper.

TABLE 6

*The effect of different metals upon the development of color in 5% lactose solutions heated to 120° C. for 20 minutes\**

METAL	COLOR	METAL	COLOR
Not any	9 $\frac{1}{3}$	Al	9 $\frac{1}{3}$
Sn	8 $\frac{1}{3}$	Pb	9 $\frac{1}{3}$
Zn	9 $\frac{1}{3}$	Hg	9 $\frac{1}{3}$
Cu	10 $\frac{1}{3}$	Ni	9 $\frac{1}{3}$
Fe	10 $\frac{1}{3}$	Monel	10

\* Phosphate buffer concentration = M/2; (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> concentration = M/42; reaction approximately constant at pH 6.18.

Solutions of 5 per cent lactose in M/2 phosphate buffer and containing a concentration of M/42 (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> were sealed in atmospheres of dif-

ferent gases. After heating, the tubes were stored for several weeks. Some of the tubes were broken open to allow the air to freely reach the solution. The results obtained are shown in table 7. The differences in color of solutions heated in an atmosphere of air, nitrogen, oxygen, carbon dioxide or in a vacuum are very slight immediately after the heating period is completed. However, these differences in color are considerably intensified when the solutions are held in storage in the presence of air or oxygen. Evidently the development of color during storage either required, or is markedly increased by, the presence of oxygen.

TABLE 7

*The effect of different gases upon color development in 5% lactose solutions heated to 120° C. for 20 minutes and stored at room temperature. Sodium phosphate buffer = M/2;  $(\text{NH}_4)_2\text{HPO}_4 = \text{M}/42$ . pH constant at 6.18*

GASEOUS MEDIUM PRESENT IN TUBE		COLOR INCREASE DURING HEATING AND STORAGE			
DURING STERILIZATION	DURING STORAGE	COLOR AFTER HEATING	1 day	8 days	3 weeks
Air	Air—sealed	9 $\frac{2}{3}$	10 $\frac{1}{3}$	14	16 +
Air	Air—open	9 $\frac{2}{3}$	11 $\frac{1}{3}$	14 $\frac{1}{2}$	
Vacuum	Vacuum—sealed	9 $\frac{1}{2}$	9 $\frac{1}{2}$	9 $\frac{1}{2}$	10
Vacuum	Air—open	9 $\frac{1}{2}$	10 $\frac{1}{3}$	14 $\frac{1}{2}$	
Nitrogen	Nitrogen—sealed	9 $\frac{1}{3}$	9 $\frac{1}{3}$	9 $\frac{2}{3}$	11
Nitrogen	Air—open	9 $\frac{1}{3}$	10 $\frac{2}{3}$	14 $\frac{1}{2}$	
Oxygen	Oxygen—sealed	10 $\frac{1}{3}$	11 $\frac{1}{2}$	14 $\frac{1}{2}$	16 ++
Carbon-dioxide	Carbon-dioxide—sealed	9 $\frac{1}{2}$	9 $\frac{1}{2}$	9 $\frac{1}{2}$	10

The temperature of storage influences to some extent, the rapidity of color development after the initial period of high heating is over. Some results obtained on tubes of lactose solutions stored at 10° C., 25° C., and 42° C., after sealing in air and heating, are given in table 8. The higher temperatures of storage caused greater darkening in color.

It was interesting to note that the color which developed in heated lactose solutions during storage was markedly browner in hue than that in solutions in which the same depth of color was developed by heating alone. This change in shade from reddish yellow to brown occurred largely during the first few days of storage. It was difficult to match the stored samples with the color standards used for the freshly heated solutions but the results given for these samples correctly reflect the order of color intensity in each, although the match with the standard was not perfect.

In view of the foregoing results, it did not appear that any normal variation in the manufacturing process of condensed or evaporated milk would

TABLE 8

*The development of color during storage of 5% lactose solutions buffered by M/2 sodium phosphate and heated to 120° C. for 20 minutes. Average initial reaction = pH 6.12; average final reaction after heating = pH 6.16*

STORAGE TEMP.	CONCN. OF $(\text{NH}_4)_2\text{HPO}_4$	COLOR DEVELOPMENT DURING STORAGE					
		Not held	1 day	4 days	12 days	25 days	60 days
° C.							
10		5	6	7½	7%	7%	7%
25		5	6	7%	7%	8	9
42		5	6	7	8	9½	11
10	M/42	9%	10%	11½	13	15	15½
25	"	9%	11	13	14½	16	18*
42	"	9%	11	13	14½	17*	20*

\* Approximate figures. No color standards available darker than 16.

eliminate color development, but it was deemed of interest to know what chemical means might be effective in preventing the appearance of color in lactose solutions during heating. A number of compounds were added to 5 per cent lactose solutions buffered by M/2 sodium phosphate and containing M/20  $(\text{NH}_4)_2\text{HPO}_4$ . Small quantities of hydroquinone,  $\beta$ -naphthol, resorcinol, catechol, and chromous chloride did not prevent the development of the usual amount of color shown by control samples heated under identical conditions.

Sodium bisulfite was very effective in preventing the development of any brown color in milk or lactose solutions during heating. At a concentration of M/24 it prevented any color development in the above lactose solution after heating to 120° C. for 20 minutes. The action of sulfite in retarding color formation in sugar syrups is well known in the cane and beet sugar industries.

Ramsey, Tracy and Ruehe (8) have observed that color formation in evaporated milk could be prevented when sufficient formaldehyde was added before sterilization. In view of the known affinity of formaldehyde for amino acids, this observation was taken as further evidence of a sugar-amino condensation reaction being entirely responsible for color formation. Some interesting data on the effect of increasing quantities of formaldehyde upon color development in lactose solutions are given in table 9. The experiments were run with and without additions of glycine to the solutions. Very small concentrations of formaldehyde markedly increase the color of the solutions while larger quantities inhibit color formation. The trend of the results appear the same whether glycine is present or absent. The action of formaldehyde appears to be more or less independent of the

TABLE 9

*The effect of formaldehyde upon the color of 5% lactose solutions heated to 120° C. for 30 minutes. Half molar concentration of maleate buffer in all solutions*

FORMALDEHYDE CONCENTRATION	NO GLYCINE		COLOR	GLYCINE CONCENTRATION = M/142		COLOR
	Reaction			Reaction		
	Before heating	After heating		Before heating	After heating	
%	pH	pH		pH	pH	
0.00	6.64	6.44	7½	6.66	6.44	10½
0.14		6.45	10		6.44	15
0.35		6.43	9		6.44	14
0.57		6.41	8½	6.66	6.40	13½
1.14		6.40	5½	6.65	6.37	11
1.60	6.67	6.40	3½	6.62	6.36	10
2.00	6.66	6.34	1½	6.58	6.29	8

amino acid content of the lactose solutions. Substantially the same results as those reported in table 9 were obtained by using sodium phosphate as a buffer in place of maleate buffer. The nature of the reactions involved here are too obscure to render attempts at an explanation profitable at this time.

The same general relations found to exist for color development in lactose solutions were found to hold true also for color formation in skim milk. However, due perhaps to the opaque nature of the milk, many of the delicate differences which are easily seen in the lactose solutions are not apparent in the milk. Pure solutions are therefore much superior to the heterogeneous mixture found in milk for a study of the basic relationships of the different components.

#### DISCUSSION

Since the hydrogen-ion concentration of a lactose solution is of great importance in color development during heating, a study of color formation must be planned to include adequate control of the reaction. From the results reported here it would appear that sodium phosphate and sodium maleate are very satisfactory buffers for maintaining the pH of a solution near to that found in milk, the phosphate radical being normally present in milk and the maleate being available for use where milk constituents are to be excluded from the solution.

The study of color development in the presence of phosphate and maleate buffers showed in three different series of experiments that the phosphate possessed a specific effect in darkening the color of heated lactose solutions. That portion of the darkening which could be attributed to

phosphate ions required the presence of appreciable quantities of phosphate, the depth of color developed being dependent upon the quantity of phosphate ions in the solution.

While it is interesting to speculate as to the nature of the reactions involved in the color development noted in this paper, there is insufficient evidence at hand to formulate any reaction mechanism. The data of table 4 show that color will form in pure lactose solutions during heating in the absence of all other materials except a maleate or phosphate buffer. This fact indicates that part of the color developed in milk during heating is due to a caramelization of the lactose.

Considering the large amount of research which has been done by many investigators it appears certain that there is a combination between amino acids and reducing sugars. The data given here show that the presence of an ammonium salt or an amino acid causes a very great darkening in the color of heated lactose solutions. Because of the limited variation in the pH of milk, and its relatively high protein content, this amino-sugar combination doubtless accounts for a major portion of the color formed in milk during heating. Since the phosphate ion concentration was found to influence color in lactose solutions and since the concentration of this ion is relatively high in milk, the contribution of the phosphate to color development is probably considerable.

The findings that the shade of color produced in storage was different from that which is formed during heating seem to indicate that two different reactions occur. Additional evidence to this effect has previously been shown in the case of evaporated milk (15) which was sterilized and held in storage. During sterilization there was a progressive change in hue, brilliance and chroma while the color change observed during storage was one in chroma only.

Results from the study of color development in lactose solutions seem to justify certain conclusions with regard to evaporated milk. Perhaps half of the color of evaporated milk as it reaches the consumer is developed during storage, the other half arising as a result of the sterilization process. The color which forms during storage may be partially or almost wholly eliminated, depending upon how brief the storage period can be made. If storage is necessary temperatures of 15° C. or lower are desirable. Where the storage temperature is not controlled, summer storage is much more detrimental to the color of the product than is storage for the same length of time during the winter.

Improvement in the color which appears during sterilization of evaporated milk is more difficult. Complete elimination of oxygen from the milk before sterilization should yield a lighter product. However the degree of improvement would probably not justify the expense of this procedure.

The addition of chemical substances which might prevent color development would of course not be permissible.

Exclusive of hydrogen-ion concentration, the lactose content of the milk and the composition of the gases present in the container, the factors which contribute to the color of milk during heating may, from the results of this investigation, be listed approximately in the order of their importance as: (1) reactive amino groups contained in the protein or arising as protein decomposition products, (2) the normal tendency of the lactose on heating at pH 6.5, to form brown lacto-caramel, (3) the phosphate content of the milk, and (4) the presence of metallic catalysts.

#### SUMMARY

1. Reproducible color standards for measuring the color of lactose solutions in which heat has produced varying shades of brown have been described and defined in numerical terms according to the Munsell system of color measurement.

2. The presence of the phosphate radical in lactose solutions during heating has been shown to exert a specific effect in causing darkening in the color of these solutions.

3. Color development in lactose solutions during heating is increased with increasing concentration of hydroxyl-ions, lactose, amino acids, ammonium salts, phosphate and oxygen. The presence of copper or iron catalyzes the color reaction while tin retards color formation. A very small quantity of formaldehyde increases color while larger amounts markedly restrict color development. Sodium bisulfite will entirely prevent the appearance of color. When amino acids or proteins are present during heating, color is probably due both to the formation of a complex material formed from the lactose and an amino group and to a polymerization of the sugar to lacto-caramel. Either reaction may occur at the hydrogen-ion concentration found in milk.

4. An effective means of preventing color development in lactose solutions during heating which would be suitable for use in improving the color which appears in evaporated milk during sterilization was not found. However, the results obtained with lactose solutions substantiate the fact that the objectionable darkening in color of evaporated milk which occurs during storage can be materially lessened by shortening the storage period or lowering storage temperature.

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## THE USE OF CITRIC ACID AND SODIUM CITRATE IN BUTTERMAKING

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The flavor and aroma of butter have always been recognized as being of the utmost importance in determining the quality of butter. Many of the larger markets demand a butter with a high flavor and aroma and to meet this demand it is necessary to make the butter from ripened cream. The acidity of such butter may be the cause of loss to the manufacturer or wholesaler due to the fact that such butter deteriorates more rapidly in storage.

During the past few years there has been considerable research carried on in an effort to isolate and identify the substances that are responsible for the flavor and aroma of butter, in order that these aroma constituents might be added in pure form to butter made from sweet cream and thereby give the butter the high flavor and aroma characteristic of ripened cream butter, without the possible danger of deterioration due to the high acidity. All of the investigators seem to agree that the major flavor and aroma constituents are methylacetylcarbinol and its oxidation product diacetyl. The amount of these constituents present in the butter varies somewhat according to the different investigators; Davies<sup>1</sup> reporting 0.05 to 0.5 parts per million, Obst<sup>2</sup> found five grams of diacetyl in 2000 pounds of butter or 5.5 parts per million, while Darres<sup>3</sup> reports that the presence of 0.000,000,2 per cent of diacetyl gives butter its characteristic aroma.

Pritzker<sup>4</sup> reports that wood vinegar is a very cheap source of these compounds as he found diacetyl in amounts varying from 0.1 to 1.92 grams per liter, and methylacetylcarbinol 0.5 to 12.0 grams per liter. Numerous other sources have been studied and these aroma constituents of butter have been found in varying amounts in such substances as coffee, cacao, and honey.<sup>5</sup>

As a result of the isolation of these flavor constituents of butter, a num-

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<sup>1</sup> Davies, W. L., *Food Manufacture* 8: 346-8 (1933); *cf.* *Chem. Abs.* 28: 835, (1934).

<sup>2</sup> Obst, W., *Molk.-Ztg. Hildesheim* No. 64, June 5, 1930, pg. 193-4; *cf.* *Milchw. Literaturb.* 3: 367, 1930.

<sup>3</sup> Darres, W. C., *Sonderdruck aus Ford Manufacture* Okt. 1933, 35; *cf.* *Milchw. Literaturb.* 3: 367, 1930.

<sup>4</sup> Pritzker, J., *Chemiker-Ztg.* No. 80, Oct. 7, 1933; *cf.* *Milchw. Literaturb.* No. 81, Feb. 28, 1934.

<sup>5</sup> Schmalfuss and Barthmeyer, *Biochem. Zeitsch.* 216: 330-5, 1929; *cf.* *Chem. Abs.* 24: 1432, (1930).

ber of artificial butter flavors have appeared on the market in an attempt to meet the demand for a butter with a high aroma and a low acidity. Such additions have been tried in some of the European countries and the general opinion of the writers is that while there is a marked improvement in the flavor and aroma of the butter, the effect is rather temporary and the diacetyl and methylacetylcarbinol may act as catalysts in hastening the deterioration of the butter that is not used within a few days after the addition of the artificial flavoring material. Another objection that is raised is that such materials may be used in margarine in an attempt to give a more exact imitation of butter.

In the isolation of these flavor constituents the first materials to be studied were the starter cultures used in the manufacture of butters with decided aromas and flavors. While the amount of material isolated from this source was naturally very small, the figures given previously indicate that only a very slight trace of diacetyl is needed to increase the aroma of butter. There is a difference of opinion as to the source of these aroma constituents, as indicated by the writings of several investigators. Cuisa<sup>6</sup> decided that the methylacetylcarbinol was formed through the action of trypsin and its enzymes on the proteins. Davies<sup>7</sup> states the carbinol is formed from the lactose, certainly the latter is more easily acted upon by the bacteria in the butter starter. Hammer and his associates<sup>8</sup> report a detailed study on the presence of methylacetylcarbinol and diacetyl in starter cultures. They show that the amount of these constituents in a culture is increased by the addition of citric acid or sodium citrate in varying amounts. Raffey<sup>9</sup> reports similar results using 0.1 to 0.4 per cent citric acid in the milk. In a recent paper Ritter and Strussi<sup>10</sup> have confirmed the above findings that the aroma of butter is due to the presence of diacetyl which is formed by the aroma producing organisms through the utilization of the citric acid. These authors also added citric acid to the cream, but in their opinion this addition resulted in the production of excessive flavor.

The addition of citric acid and sodium citrate to starter cultures was investigated by Templeton and Sommer.<sup>11</sup> It was found that the addition of 0.2 per cent of citric acid or its equivalent as sodium citrate to the milk to be used in the starter culture increased the volatile acidity approximately 50 per cent, while the titratable acidity increased only 10 per cent. While there is no direct evidence that the volatile acids are responsible for the

<sup>6</sup> Cuisa, W., *Ann. chim. applicata* 22: 747-53 (1932); *cf.* *Chem. Abs.* 27: 1897 (1933).

<sup>7</sup> Davies, W. L., *Food Manufacture* 8: 346-8 (1933); *cf.* *Chem. Abs.* 28: 835 (1934).

<sup>8</sup> Hammer, B. W., Michaelian, M. B., and Farmer, R. S., *Iowa State College of Agriculture Research Bulletin* No. 155.

<sup>9</sup> Raffey, O., *Osterr. Milchwirtschaft. Ztg.* 20: 250-1 (1932); *cf.* *Chem. Abs.* 27: 2735 (1933).

<sup>10</sup> Ritter, W., and Strussi, D., *Schweiz. Milchztg.* 60: 47-8 (1934); *cf.* *Chem. Abs.* 28: 4494 (1934).

<sup>11</sup> Templeton, H. L., and Sommer, H. H., Jr. *DAIRY SCIENCE* 12: page 21, 1929.

characteristic flavor and aroma of butter or starter cultures, they no doubt play some part either as intermediates or in establishing conditions which favor the growth of the aroma producing organisms. There is evidence that high volatile acidity may be indicative of a high diacetyl and methyl-acetylcarbinol content of the starter culture and the butter made from such a starter.

These findings indicate quite definitely that the flavor and aroma of butter may be increased by the enrichment of the citrate content in the starter or cream. The fact that milk is known to be quite variable in its citrate content may be regarded as further justification for such a practice. This practice of supplementing the natural composition of the milk is now definitely established in the evaporated milk industry, where it is frequently necessary to add citrate or phosphate to control the stability.

The results of the researches to be presented in this paper are a continuation of the previous work, since the starters studied earlier were used in the making of the butter to be described at this time. These were regular commercial starter cultures propagated in both sterilized and pasteurized milk for more than two months before the buttermaking studies were started. Eight of these cultures were used, and a series of experiments were performed with each one using different treatments of the cream and starter, as will be given in detail in connection with the tables. In each series of experiments there was one sample of butter that was made with the culture alone, without the addition of either citric acid or sodium citrate to the culture at any time. This was the control sample for each series of experiments.

The butter made in this study was scored as fresh butter within ten days after making, again after six weeks' storage at 40° F. (4.40° C.), and after six months' storage in an ice cream hardening room where the average temperature was approximately 06° F. (-17.78° C.). The butter was packed in carefully cleaned half-pint Mason jars and three jars were filled from each churning to insure an unused sample for each scoring. The men requested to score the butter were competent judges, and the samples were numbered in such manner that the same treatment did not always have the same number. In some cases the judges did their scoring as a group, while other scorings were made by each judge on different days. Sixty-five samples of butter were made on a laboratory scale using a small hand churn of the kangaroo type. The butter was worked in a small home-made worker equipped with rolls similar to those found in large churns. In order to insure as uniform a body as possible, the butter was all churned at approximately the same temperature and received the same amount of working. Two per cent of salt was added to the butter before working. As indicated previously, the butter was packed in Mason jars and these in turn were kept in cardboard boxes to exclude light.

In table No. 1 the capital letters indicate the starter that was used. The

cream used in this series of experiments was taken directly from the separator, pasteurized at 140° to 145° F. (60.00° to 62.78° C.) for 30 minutes and then cooled rapidly to 70° F. (21.11° C.). At this temperature the cream was inoculated with the starter using about 5 per cent, and the citric acid or sodium citrate added in amounts indicated. After thorough mixing of the sample the titratable acidity was determined and all samples having 0.35 per cent or more calculated as lactic acid were immediately cooled to 45° to 50° F. (7.22° to 10.00° C.) and held at this temperature until churned. The remaining samples were kept in a water bath at 70° F. until the titratable acidity was .35 per cent. This holding time varied from six to ten hours although a few samples required a somewhat longer time. The cream was chilled for at least four hours before churning, the maximum time being twelve hours. In the series of experiments marked with a single letter, the cream used tested about 30 per cent butterfat, and four and one-half pounds of cream were used for each sample.

In those series of experiments marked with the double letters, the starter used was the same as in the corresponding single letter, but only one liter of 55 to 60 per cent cream was used for each experimental churning. This cream received the same treatment as the other cream except that when the cream had been cooled to 70° F., 750 cc. of the starter culture were added to each liter of cream with the other additions as indicated in table 1a. The cream and starter were thoroughly mixed by shaking. As the titratable acidity in each case was over 0.35 per cent the samples were immediately cooled to 50° F. and placed in storage. These samples were scored at the same ages and by the same judges as the other samples.

The explanation of the terms used in the treatment of the different samples is the same as in the paper on starters, namely—fresh citric acid or sodium citrate indicates that the additions were made to a control culture 24 hours previous to the inoculation of the sample of cream; an old citric acid or sodium citrate culture is one that has been carried through a number of transfers, with citric acid or sodium citrate added to the culture at each transfer before the inoculation of the sample of cream. When the term "control" is used, that indicates that the starter added did not contain any added citric acid or sodium citrate.

In tables No. 1 and 1a, the average scores of each sample are given at the three scoring ages. It would be impossible to present the individual scores and the comments of the judges on all the samples of butter, so these summary tables are presented. In the series with starter culture "A" two sample jars were broken after the first scoring so that the six weeks' scoring was omitted in two instances. Table No. 2 is based on the preceding tables, in that the samples of butter are ranked in order of their scores within the series with each starter culture, and these rankings are compiled as shown. Tie scores were broken in favor of the sample having the highest score from one of the judges, or if that were impossible on the basis of the criticisms

*Average scores of butters made with additions of citric acid and sodium citrate*

TABLE 1

Media/Inoculum	A		B		C		D		E		F		G		H		I		J	
	Frash	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.	6 mts.
Control	91.13	90.75	91.13	89.47	89.43	91.25	91.33	89.25	91.50	91.33	89.25	91.50	91.33	89.25	91.50	91.33	89.25	91.50	91.33	89.25
Control + .25 Citric Acid																				
Control + .50 Citric Acid																				
Control + .75 Citric Acid																				
Frash Citric Acid Culture	90.86	91.50	89.80	91.25	90.15	90.75	90.50	90.06	91.00	91.57	90.25	90.98	91.47	91.13	90.75	90.50	89.86	90.75	90.50	89.86
Frash Citric Acid + .25 Citric Acid																				
Frash Citric Acid + .50 Citric Acid																				
Old Citric Acid + .25 Citric Acid	90.79	90.17	90.63	91.19	89.25	90.00														
Old Citric Acid + .50 Citric Acid																				
Frash Sodium Citrate Culture																				
Frash Sodium Citrate Culture + .25 Sodium Citrate	91.08	91.00	90.88																	
Frash Sodium Citrate Culture + .50 Sodium Citrate	91.25	90.81	91.25	89.85	90.75															
Old Sodium Citrate Culture																				
Old Sodium Citrate Culture + .25 Sodium Citrate	91.45	90.75	91.00	91.15	90.25	90.19	91.13	91.45	91.75	91.13	91.42	91.25								
Old Sodium Citrate Culture + .50 Sodium Citrate																				
Control	91.50	90.86	90.49	91.14	89.64	90.10	91.19	90.64	89.58	91.84	91.57	89.94	91.19	90.64	90.18	91.25	90.25	89.81	91.38	90.47

Figures in brackets indicate number of scores averaged

*Average scores of butters made with additions of citric acid and sodium citrate*

TREATMENTS	AA			ES			EH			AVERAGE		
	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.
Control	91.25	90.00	90.44	91.25	91.35	89.68	91.56	91.85	90.80*	91.80	91.08	90.19
Control + .25 Citric Acid	92.13	89.85	89.85	91.85	91.00	89.75	91.25	92.08	91.19	91.84	90.97	90.19
Control + .46 Citric Acid	92.13	90.00	89.80	92.13	92.50	89.69	92.00	91.56	90.69	92.08	91.26	89.96
Old Citric Acid + .25 Citric Acid	90.65	90.85	90.06	91.25	90.35	90.50				90.94	90.35	90.28
Control + .25 Sodium Citrate	91.15	90.75	89.00							91.15	90.75	89.00
Control + .25 Sodium Citrate + .25 Citric Acid							92.51	92.85	92.00	92.81	92.85	92.00
Old Sodium Citrate + .25 Sodium Citrate	91.25	90.17	90.15	91.65	91.67	90.28	92.25	92.08	90.98	91.71	91.31	90.46
Old Sodium Citrate + .46 Sodium Citrate				91.65	92.00	90.56	91.66	92.00	90.25	91.75	92.00	90.51
	91.48	90.18	89.75	91.65	91.47	90.06	91.95	92.07	90.92	91.86	91.24	90.25

given. From this table it is evident that in the single letter series there were two or more samples that were preferred to the control sample in all but one instance. The column at the right of this table gives for each treatment the percentage of samples that ranked third or better. In the double letter series there were two instances in which the control sample was either first or second, and it was below third in all the others.

On the basis of the first two scorings of this butter it seemed advisable to do further work on a larger scale. The second series of experiments was conducted on cream arriving at the University creamery at a time when the amount of cream varied rather widely from day to day and the acidity was usually rather high. Most of the cream was neutralized to 0.25 per cent acidity with a commercial sodium bicarbonate neutralizer before pasteurization. When there was sufficient cream it was divided into two portions. After cooling to 70° F. one vat was inoculated with the starter culture to act as the control, and citric acid or sodium citrate added to the other in the amounts shown in table No. 3. The butter was made by the regular buttermaker under the conditions usually existing in the plant. When the butter was removed from the churn three half-pint Mason jars were filled as samples for the judges to score. The samples were scored as fresh butter and again after six weeks and six months. The first five lines of table No. 3 represent a portion of the results obtained in this second series of experiments. All of these were made from starter culture "E" except the one in which the citric acid was added to the cream without starter. The average scores of these samples by the same judges are given in the table. In connection with this series of experiments the fat losses in the buttermilk were studied, using the Butyl alcohol modification of the Babcock test. The results showed that the treatment received by the cream did not increase the fat losses in the buttermilk. In some instances there was a decided reduction in the fat loss.

As a further confirmation of these results, another series of commercial churnings were made and two samples of each were submitted to the same

TABLE 3

TREATMENT	FIRST			SECOND			THIRD			FOURTH			FIFTH			SIXTH			Above Fourth Place
	Fresh	6 Mo.	6 Mo.	Fresh	6 Mo.	6 Mo.	Fresh	6 Mo.	6 Mo.	Fresh	6 Mo.	6 Mo.	Fresh	6 Mo.	6 Mo.	Fresh	6 Mo.	6 Mo.	
Control # 1			1				2	2	1	1	2	2	4	2	1	1	2		25
Control + .05 Citric Acid	2	1	2	1	1		1			2	1	1		1	2	1	1		44
Control + .05 Citric Acid											1					2	2	2	20
Fresh Citric Acid Culture	1	1		1	2		1	2		2			2			1	1	1	56
Fresh Citric Acid + .05 Citric Acid	1			1	1	1				1				1					57
Fresh Citric Acid + .05 Citric Acid				1	1		1												100
Old Citric Acid + .05 Citric Acid	1	1	1				1	1	1										100
Old Citric Acid + .05 Citric Acid				1			1			1	1	2		1	1	1			22
Fresh Sodium Citrate Culture		1	2	1	1					1		1		1		1			56
Fresh Sodium Citrate Culture + .05 Sodium Citrate				1	1	1	1						1					1	67
Fresh Sodium Citrate Culture + .05 Sodium Citrate	1		1	2	1				2	1	1	1				1			67
Old Sodium Citrate Culture		1	1							1									67
Old Sodium Citrate Culture + .05 Sodium Citrate		1		1		1			1	1				1					67
Old Sodium Citrate Culture + .05 Sodium Citrate	2	2	1		1	2	2	1	1		1		2		2		1		67

Control # 3a			1	1					1	1		1	2	1			1	72
Control + .05 Citric Acid	1				1		1				2	1	1		1	1		53
Control + .05 Citric Acid	1	1		1		1				1	1			2		1		64
Old Citric Acid + .05 Citric Acid			1		1			1						2	1			80
Control + .05 Sodium Citrate		1										1					1	33
Test # 1 0.05 Sodium Citrate + .05 Citric Acid	1	1	1															100
.05 Sodium Citrate + .05 Sodium Citrate				1	1	2	1	2	1	1								88
.05 Sodium Citrate + .05 Sodium Citrate					1		1		1	1	1						1	80

\* Each treatment (sample) was a member of a series of six samples. Each treatment was represented several times (see Tables 1 and 1a) in such six sample series, according to the number of original starter cultures with which the treatment was tried. At each scoring each treatment could rank anywhere from first to sixth place. The table records the number of times the respective treatments ranked first, second, third, fourth, fifth and sixth. The final column is explained by the following illustration:—Control No. 1 is represented 23 times in first to sixth rank, but only six times in the first three places. Therefore, it placed third or better in only 26 per cent of the placings.

judges that had scored the others, as well as to a number of other men in different parts of the country. These samples were scored as fresh butter and again after six months storage. As this work was done in August and the butter had to be shipped some distance in small cardboard boxes, it is possible that the score that the butter received might have been higher if shipping conditions had been better. The scores of these samples of butter were often times very divergent, ranging from 87 to 92 on samples of butter from the same churning. The last three lines of table No. 3 give the aver-

TABLE III  
*Average scores of butters made with additions of citric acid and sodium citrate*

CONTROL BUTTER SCORES			TREATED BUTTER SCORES			TREATMENT
Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	
91.75	90.58	90.67	92.08	91.00	91.50	Fresh Citric Acid Culture
			89.67	90.00	90.33	Fresh Sodium Citrate Culture
91.83	90.08	90.33	91.08	89.50	89.33	No starter + .075% Citric Acid Added
91.83	89.75	88.83	90.96	90.00	90.33	Control + .1% Citric Acid as Sodium Citrate
			91.46	90.25	90.67	Control + .1% Citric Acid
90.63		89.91	90.67		89.87	Fresh Citric Acid Culture
89.79		88.08	89.90		89.88	Control + .1% Citric Acid as Sodium Citrate
91.19		89.42	90.03		88.96	Old Sodium Citrate Culture + .1% Citric Acid as Sodium Citrate

age scores of the eleven men examining these samples of butter. The first two samples were made with starter culture "E" and the last one with "H."

While the results of these later series of experiments do not show the same degree of improvement of the treated samples over the controls there is evidence that the addition of citric acid or sodium citrate to the starter culture, the cream to be used for the buttermaking, or both, gives the resulting butter higher flavor and aroma.

#### SUMMARY

This work shows that the addition of citric acid or sodium citrate to either cream or the starter or both tends to produce a butter of more desirable flavor and aroma than the untreated butter made from the same cream.

The findings of previous investigators concerning the effect of high acidity on the keeping quality has been confirmed in some of the samples of butter scored, while in other instances there is very little difference in the scores of the fresh butter and the score after storage. In numerous samples the score after six weeks' storage was higher than that given the fresh butter.

It was noticed that the use of citric acid tended to lower the fat losses in the buttermilk.

In flavor and aroma the buttermilk from the treated cream compared very favorably with the starters.

The addition of citric acid or its equivalent in sodium citrate in amounts not to exceed 0.2 per cent of the weight of the cream is sufficient to produce an increase in the score of the butter made from the cream. Their addition in the starter only, is noticeable in the score of the butter.

In conclusion the authors wish to express their thanks to Charles Pfizer and Company, Inc., for the fellowship that has made this work possible, and to those gentlemen who gave their time and services in the scoring of the samples of butter.

## THE ACTION OF MILK FAT AS A FOAM DEPRESSANT

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The action of lipid substances as foam deterrents is a well-known phenomenon. Eichholtz (1) concluded that the action of these substances was due to the removal by them of the protein adsorbed at the air-solution interface. Van Dam (2) attributed the destructive action on foam of the fat globules in milk to the distortion produced in a thin film when the thickness of the film approached the dimensions of the globules. Sommer and Horral (3) assumed that the reduction in foaming power occurred because the serum did not adhere to the fat globules with a force equal to the cohesion of the serum for itself, as a consequence of which a point of weakness existed in the neighborhood of the fat globules. King (4) recently observed by means of a microscope the changes occurring in skim milk foam when the fat contained in whole milk is permitted to act on it. He suggested as a possible explanation of the observed changes the lowering in surface tension due to the formation of a fat film. Reference was made in the publication to the work of Mohr and Brockman (5) who observed a lowering in superficial viscosity corresponding to an increase in the fat content and to a decrease in the foaming power of milk.

The results of investigations in this laboratory on surface phenomena in milk products are concerned in part with the views expressed by the various authors quoted above. These results will be presented and discussed in this paper.

### *Experimental*

In the following experiments the surface properties of milk serum prepared from skim milk by ultrafiltration were compared with the surface properties of milk serum containing one per cent by volume of added skim milk. The addition of the skim milk to its serum produces a marked decrease in the foaming power of the serum, an effect which may be demonstrated to be due to the minute lipid content of the resulting suspension.

The following measurements were made:

- (1) Foaming power
- (2) Surface tension (dynamic and static)
- (3) Superficial viscosity
- (4) Adsorption of nitrogenous material

**Measurement of Foaming Power:** To measure foaming power, 20-cc. samples were measured into graduate cylinders and agitated, uniformly, in

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pairs to give relative values for the quantity of foam remaining one minute after agitation has ceased. Typical results obtained by means of this procedure are included in table 1.

TABLE 1

*The dynamic and static surface tension values of water and milk serum solutions. The relative foaming capacities of these solutions*

SYSTEM	SOLUTION	RELATIVE FOAMING CAPACITIES	DYNAMIC VALUES (JET METHOD)	DYNAMIC VALUES (RING METHOD)	STATIC VALUES (RING METHOD)
Serum	Water	1.0	<i>dynes/cm.</i> 72.0	<i>dynes/cm.</i> 72.0	<i>dynes/cm.</i> 72.2
	Milk serum		72.0	61.3	51.1
	Milk serum + 1% skim milk	0.5	72.0	61.3	50.7

Measurement of Surface Tension: Static surface tension measurements were made by the ring method. Dynamic measurements were made according to the method of capillary jets, as described by Rayleigh (6) and according to a procedure based on the ring method and differing from it only in that the surface of the solution under investigation was continuously renewed during a measurement by the slow and controlled overflow at the surface of the solution.

The measurement by the method of capillary jets furnished information concerning the tension of surfaces 1/200 second old, while the modified ring method permitted the measurements of dynamic tension corresponding to a somewhat older surface.

Typical results are given in table 1. The values given for the dynamic surface tension as determined by the method of capillary jets represent merely those calculated relative to that of water on the basis that the tensions vary inversely as the square of the wave-length of the jets.

The differences in the jet characteristics studied bear no correspondence to differences in foaming power. Thus, both milk serum and the serum containing skim milk show the same dynamic surface tension as pure water. Neither the dynamic, nor the static values as determined by the ring method show any significant differences in the tensions of the two solutions. Consequently, contrary to the ideas expressed by King, it is hardly likely that the action of the fat in milk as a foam deterrent is to be correlated with its action as a surface tension depressant.

Measurement of Superficial Viscosity: Superficial viscosity determinations by the method depending upon observations of the damping of a torsional pendulum yielded values for milk serum, and for the serum containing skim milk, which were much greater than the value for pure water. No significant difference, however, existed between the surface viscosities of the

two solutions. The data pertaining to these measurements are omitted. It was found difficult to obtain reproducible results by this method, probably because of difficulty in the production of surfaces of the same age. In order to avoid this difficulty, a procedure was developed depending upon the impedance offered to the rise of small bubbles through a slightly inclined tube containing the solution under investigation. The ratio between the time required for a bubble to travel one centimeter, in the solution under consideration, and the time required for a bubble of the same size to traverse the same distance in a sucrose solution of the same bulk viscosity and of approximately the same density as that of the solution under investigation, was arbitrarily taken to represent an index of the magnitude of viscous forces localized on the surface of the bubble. The indices obtained in this manner are given in table 2. The size of the bubble, and the bulk viscosity, and density of the sucrose solution require control, inasmuch as they are factors influencing the rate at which the bubble rises.

TABLE 2

*The viscosity and superficial viscosity indices of various reference solutions, and of milk serum solutions, as determined by observations of the rate of rise of small bubbles*

SYSTEM	SOLUTION	RADIUS OF BUBBLE	VELOCITY OF BUBBLE	SUPERFICIAL VISCOSITY INDEX	RELATIVE VISCOSITY 30° C. H <sub>2</sub> O = 100
		cm.	cm./sec.		
Reference Solutions	Water	0.25	4.31		1.00
	7% sucrose	0.25	3.30		1.16
	11% "	0.25	2.90		1.32
	20% "	0.25	2.50		1.88
	30% "	0.25	1.88		2.98
	40% "	0.25	1.17		5.48
Serum	Milk serum	0.30	1.41	2.34	1.16
	Milk serum + 1% skim milk	0.30	1.71	1.93	1.16
	Serum + 2% skim milk	0.31	1.84	1.79	1.16

The results indicate that the addition of skim milk to milk serum produces a decrease in the superficial viscosity index of the serum. This change may possibly be due to the lubricating action of the fat. However, it is doubtful whether this decrease in the superficial viscosity index is sufficient to account for the marked differences observed in foaming power inasmuch as the superficial viscosity of the serum, even after the addition of milk, is quite marked. Supporting this conclusion is the observation (date not recorded in this paper) that the superficial viscosity of a dilute sodium oleate solution of marked foaming power is the same as that of a suspension

of weak foaming power, containing free oleic acid in addition to sodium oleate.

In contradistinction to the results of superficial viscosity measurements by the observation of the damping of a torsional pendulum, the results of superficial viscosity determinations on sodium oleate solutions by the method described above indicate the existence of a viscous force considerably greater than that belonging to pure water.

*Adsorption Determinations:* The method used to measure adsorption was similar to that employed by McBain (8) and his collaborators. Bubbles uniform in size of measured volume and area were removed after their passage through a slightly inclined tube containing the solution under investigation; and from determinations of the weight of the solution resulting from the collapse of the bubbles, and of the concentration of the nitrogenous material in this solution, in the original solution, and in the residual solution, the quantity of nitrogenous material adsorbed per square centimeter of surface was calculated.

Nitrogen determinations were made by means of the Arnold-Gunning modification of the Kjeldahl method. All samples were run in duplicate. Distillation was effected by means of steam according to the method described by Allen in which the essential features of the micro-Kjeldahl apparatus are utilized for ordinary Kjeldahl determinations. In order to obtain a sharp endpoint, the distillate before titration was reduced in volume to 50 cc. by distillation under vacuum. The mean deviation of 42 determinations approximated 0.002 mgs. of nitrogen per gram of sample.

The adsorbed nitrogenous material was assumed to consist entirely of lactalbumin, and calculations of the weight of adsorbed material were made on this basis. The total area of the films was calculated upon the assumption that the individual bubbles were spherical. However, the shape was more nearly that of a distorted ellipsoid, and consequently the values calculated for the quantity of adsorbed material should be considered higher than those corresponding to the actual shape. For comparative purposes such as these experiments are intended, absolute adsorption values are of no particular significance. It is only necessary that the bubbles in the various solutions should be uniform in size inasmuch as Gans and Harkins (9) have shown that the degree of adsorption varies with the size of the bubble.

### *Results*

The results given in table 3 indicate that over a wide range of dilutions, the adsorption values of milk serum are nearly constant, and hence that the adsorption film may be considered as saturated even in the most dilute

TABLE 3  
*The degree of adsorption of protein from milk serum and from milk serum containing 1 per cent by volume of skim milk*

SOLUTION	1		2		3		1 MILK SERUM + 1% SKIM MILK	
	Mean of 2 expts.	Mean of 4 expts.	Mean of 2 expts.	Mean of 3 expts.	Mean of 2 expts.	Mean of 3 expts.	Mean of 2 expts.	Mean of 3 expts.
Dilution—								
pts. H <sub>2</sub> O per 100 pts. serum	0.00	66.7	900		0.00	66.7		100
Conc. N. original sol. mgs.	0.580	0.437	0.0910		0.702	0.519		0.394
Conc. N. residual sol. gm.	0.568	0.422	0.0822		0.688	0.500		0.386
Conc. N. foam gm.	0.601	0.458	0.1132		0.724	0.543		0.410
Wt. of foam gm.	63.88	55.92	70.91		66.71	52.49		74.71
Area of bubble cm <sup>2</sup>	3.38	3.87	3.80		3.42	3.65		3.37
Total area cm <sup>2</sup>	61,330	61,380	80,210		63,200	70,400		58,600
Adsorption of protein gms./cm. <sup>2</sup>	$1.8 \times 10^{-7}$	$1.7 \times 10^{-7}$	$1.5 \times 10^{-7}$		$1.9 \times 10^{-7}$	$1.6 \times 10^{-7}$		$1.6 \times 10^{-7}$

serum solution. Upon the addition of 1 per cent by volume of skim milk the surface remains saturated with respect to the quantity of adsorbed nitrogenous materials; and consequently the decrease in the foaming power of the serum cannot be explained on the basis of a change in the closeness of packing of the adsorbed molecules, in substantiation of the results obtained by means of surface tension measurements, and decidedly in conflict with the views expressed by Eichholtz.

### *Spreading Power and Foaming*

The change in the foaming power of the serum may be demonstrated to be due to the introduction of milk fat. Concomitantly forces evidently originate tending toward the destruction of a liquid film. The theories advanced by Van Dam and Sommer and Horral, as described in the introduction, are concerned with these destructive forces. That proposed by Van Dam leads to the conclusion that the destructive action should be independent of the spreading power of the emulsified fat or oil; that proposed by Sommer and Horral suggests that the less the tendency of the fatty material to spread the greater its tendency to destroy the film. Both theories lead their respective authors to the conclusion that the coarser the dispersion formed from the lipoid substances the greater the destructive action of this material. That these theories are open to criticism may be demonstrated by a simple experiment. Thus, Squibbs mineral oil, which does not spread on water and which forms a coarse dispersion with milk serum, exerts only an insignificant depressant action on the foaming power of the serum in contradistinction to the marked depressant action of butterfat, oleic acid, or triolein, substances which spread on water and which form fine dispersions with milk serum.

Edser (10) has pointed out the resemblance existing between the spreading of a rupture in a soap bubble and the spreading of a film of grease over pure water. The contraction of the soap bubble due to the spreading of a rupture has its analogue in the contraction of the surface of pure water due to the spreading of a film of grease. This analogy suggests that the presence in a thin liquid film of a substance which will spread may be considered tantamount to the existence of a rupture with the obvious exception that in the case of the spreading substance a thin film of the substance would remain, the stability of which would be such that it would also vanish quite readily. Where the size of the globule of spreading substance is insufficient to displace the entire film, a region of weakness would exist in that portion of the film displaced by the spreading substance.

The rupture of a highly viscous lamella of ice cream mix may be observed to spread quite slowly. If a drop of linseed oil is placed on such a lamella the spreading of the oil occurs at a slow rate, and the displacement

of the entire lamella by the oil may be observed. In this manner the comparatively stable ice cream mix lamella is replaced entirely by an unstable oil lamella.

It is proposed that the observed decrease in foaming power occurring when skim milk is added to milk serum is capable of interpretation on the basis of the equivalence noted between a rupture and the presence of a spreading substance in a thin film. The contractile forces arising with the formation of a liquid film, as a result of the tendency of the fat globules to spread, effect the destruction of the film in much the same way as a mechanical rupture.

It is interesting to note that the foaming power of milk decreases with an increase in temperature from 80° C. to 20° C. King (11) has recently presented evidence to show that the portion of the surface of milk covered with a film of fat increases with a rise in temperature from 10° C. to 20° C. These results, therefore, indicate that the decrease in foaming power may be explained on the basis of the increase in the tendency of the fat to spread as the temperature rises.

#### SUMMARY

The action of the milk fat in milk as a foam depressant has been discussed in connection with the theories which have been proposed to explain the phenomenon. The consequences of these theories have been investigated experimentally. Contrary to some of the ideas expressed adsorption experiments indicate that the action of milk fat is not due to the removal by the fat of the protein adsorbed at the air/milk serum interface; and surface tension, and superficial viscosity measurements indicate that the action is not attended by any significant changes in the dynamic and static surface tension, or in the superficial viscosity of milk serum.

The destructive action of milk fat and other lipoids on foam depends in some way upon the ability of these substances to spread on pure water, and consequently the theories relating to the destructive action of fat on foam which do not take into consideration the importance of spreading, are open to criticism.

It has been pointed out that the presence in a thin film of a globule of a substance which will spread may be considered tantamount to the existence of a rupture, and the suggestion has been made that the decrease in foaming power occurring when skim milk is added to milk serum is capable of interpretation on the basis of the equivalence noted between the existence of a rupture, and the presence of a spreading substance in a thin film.

Procedures have been described for the measurement of dynamic surface tension, and of an index of superficial viscosity.

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## THE DISTRIBUTION OF PHOSPHOLIPOIDS IN CREAM

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The variation of phospholipoids with the fat contents of creams, their probable relationship to the colloidal systems of milk products and their concentration to effect the presence of maximum quantities of a certain definite form of assimilable phosphorus were the chief considerations leading to this investigation.

In spite of the accepted recognition of the excellent emulsifying and other properties of lecithin and its allied phospholipoids rather little is found in the literature relative to their physical-chemical functions in milk and cream systems. Palmer and Wiese (1) (2) recently, in studying substances adsorbed on the fat globules in cream, substantiated evidence indicating that phospholipoids together with a mixture of certain proteins are invariably present as a stabilizing mixture in conjunction with the fat globule, and found that artificial milks prepared by dispersing milk fat in an aqueous suspension of (egg-yolk) phospholipoid yielded cream which separated more nearly like natural cow's milk than like milk fat dispersions having buttermilk, calcium caseinate, lactalbumin, and globulin respectively in the stabilizing sols. This observation indicates the possible importance of the presence of lecithin in milk and cream with regard to its serving to assist in the maintenance of the colloidal system.

The results of previous investigators indicate that the distribution of phospholipoids in milk products varies rather widely. The amount present in milk is reported to vary from 0.040 to 0.117 per cent. Bordas and de Raczkowski (3) found 69 per cent of the total phospholipoids passing into the cream upon separation. Gilkin (4) substantiates this and shows that about 3 per cent is retained by the skim-milk fraction while Dornie and Daire (5) report larger amounts to be retained in the skim milk. The separator slime (6) was found to comprise an appreciable amount of phospholipoids—about 12.5 per cent. When cream is churned phospholipoids are removed and the resultant butter contains lesser quantities than does the original cream (2) (7) (8) (9). These quantities, however, are appreciable and not mere traces as reported by some authors. Cusick, whose results are cited by Supplee (9), reports 0.0723 per cent di-stearyl lecithin for raw sweet butter, but when his values are recomputed on the basis of his recovered lipid phosphorus they are found to be actually twice the reported amounts or approximately 0.14 per cent. Smith's value for lipid phosphorus in sweet butter (10) is found to be equivalent also to 0.14 per cent of di-stearyl lecithin, thus checking Cusick's corrected value.

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Wiese, Nair and Fleming (11) maintain that the phospholipoid content of cream is very uniform and, working with 40 per cent cream, found it to be equivalent to 8 mg. of phosphorus per 100 grams for summer cream and 7.2 mg. per 100 grams of winter cream; and, computing their values on the basis of total fatty extract, report 19.9 and 18.2 mg. per 100 grams, respectively. Their statement is based on results obtained with cream of a definite unvarying fat content and probably produced under the same uniform conditions and, as will be demonstrated later in this work, cannot be applied to cream in general.

Observations made in this laboratory during the preliminary examination of creams of varying fat concentrations for their lipid phosphorus content, revealed that a more or less definite relationship seemed to exist between the two components. In general, it appeared that the phospholipoid content increased almost uniformly with the fat content until a certain point of fat concentration was reached when it began to diminish appreciably. On the basis of the fatty extract there was an obvious decrease in lipid phosphorus with the increase of the fat content of the cream. In table 1 are shown the lipid phosphorus and fat values obtained during the preliminary examination of pasteurized market milk and cream

TABLE 1  
*Fat and lipid phosphorus contents of market creams*

SAMPLE	FAT	LIPOID PHOSPHORUS	LIPOID PHOSPHORUS IN FATTY EXTRACT
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg per 100 grams</i>
1*	4.02	1.14	28.4
2	16.33	3.1	19.0
3	32.68	5.4	16.6
4	34.90	5.7	16.3
5	35.58	6.2	17.5
6	41.24	6.0	14.5
7	43.49	5.7	13.1
8	47.30	5.6	10.9

\* Milk.

These few preliminary values present the possibilities of an interesting picture. The phospholipoid increase on the basis of the cream as such with the increase in fat and the decrease on the basis of the fatty extract established the fact that a certain loss of phospholipoid takes place as creams of increasing fat contents are produced. In short, a great portion of the phospholipoid content is removed with the skim milk.

#### EXTRACTION METHODS

It has been found that in the fatty residues obtained by the Mojonner modification of the Roese-Gottlieb procedure for the determination of fat in

milk products are included also the milk phospholipoids. For this reason it has been recommended that these residues be employed for the determination of lipid phosphorus (10), (12), (13). Wiese, Nair and Fleming (11) with this method were able to recover quantitatively known amounts of lecithin in simple aqueous dispersions and also in the presence of milk fat. Chapman (12) experienced difficulty in the recovery of added amounts of lecithin in butterfat. The writer (14) found that slightly higher results could be obtained by employing a mixture of absolute alcohol and chloroform as the initial solvent but a persistent fluorescence carried through to the final solutions of the fatty extracts indicated possible contamination with phosphorus in other forms of combination and the method was temporarily discarded.

Holm, Wright and Deysher (19), using a method dependent upon the solvent action of a mixture of 3 parts of 95 per cent alcohol and 1 part of ethyl ether with no provision for subsequent purification of their extracts to remove phosphorus from inorganic and other sources secured results indicating the presence of phospholipoids to the extent of from 0.152 to 0.158 per cent in milks of from 2.5 to 4.3 per cent fat content. In creams having from 27 to 42 per cent fat content they report phospholipoid values of from 0.224 to 0.284 per cent. In the entire range of milk and cream of from 2.5 to 42 per cent fat contents their reported values increase from 0.152 to 0.284 per cent while the writer, working with purified extracts secured results of from 0.03 to 0.17 per cent from the same range of fat contents. The values obtained with the purification step increase from approximately 20 per cent to 61 per cent of the values reported by Holm *et al.*, thus approaching agreement with increasing fat content and attendant decreasing moisture. Obviously the presence of small quantities of moisture in the Holm extraction procedure from the source of the alcohol as well as from the milk or cream itself allows the solution of considerable phosphorus other than that of the phospholipoids. Extracts made by the writer when applying this procedure to milk continued depositing solid material despite repeated filtration for about 24 hours losing up to 17 per cent of their original phosphorus. One extract yielding 0.173 per cent of phospholipoids upon immediate analysis filtered 24 hours later gave only 0.144 per cent. Another sample of the same milk to which was added 0.3 per cent of the mixed inorganic phosphates normally present in milk yielded 0.280 per cent of phospholipoids and when filtered 24 hours later only 0.209 per cent. Still another sample of milk showing 0.028 per cent of phospholipoids by the Mojonner modification of the Roesse-Gottlieb extraction, drying and re-extraction with chloroform, gave 0.178 per cent by the Holm procedure. Addition of 0.1 per cent of  $\text{KH}_2\text{PO}_4$  increased the latter value to 0.199 per cent and 0.065 per cent  $\text{K}_2\text{HPO}_4$  added to another portion increased it to 0.196 per cent while the original value of 0.028 per cent remained unaltered

by these additions. The moisture present in the extracting mixture of the Holm procedure evidently carries with it inorganic phosphorus and thus yields high phospholipoid values.

It was finally decided most convenient and satisfactory to employ the Mojonnier modification of the ammoniacal Roesse-Gottlieb procedure for the extraction of the total fat and lipoids. Three extractions were made using a ten-gram sample and the combined solvent was evaporated to dryness on a steam bath in a tared 150 cc. fat flask with the aid of a gentle current of air from an electric fan. The fatty extract was then dried in an oven at 100° C. to constant weight (60 to 90 minutes) and the percentage of total fat calculated.

#### COLORIMETRIC DETERMINATION OF LIPOID PHOSPHORUS

The fatty residue as obtained above was dissolved in a small amount of chloroform and transferred by means of small portions of the solvent from a wash bottle to a 50 cc. volumetric flask through a small funnel containing a closely packed extracted pledget of cotton. The volume was then adjusted to the mark and suitable aliquots were removed for the determination of the phosphorus.

An aliquot containing about 0.10 mg. of phosphorus was transferred to a small flat-bottomed platinum dish and evaporated to dryness on the steam bath together with 1 cc. of a 50 per cent alcoholic solution of magnesium nitrate with the aid of a gentle current of air from an electric fan. The dish with the residue was then placed on a hot plate and the heat gradually increased until a dry charred mass resulted. The char was finally ignited to a white ash in a muffle at about 500° C. The phosphorus was determined in the residue according to Smith's modification of the Bell-Doisy procedure for blood phosphorus (10). After moistening with a few drops of distilled water 2 cc. of diluted sulphuric acid (1+1) were added and complete solution was effected. The perfectly clear and colorless resulting solution was then carefully transferred to a 200 mm. test tube calibrated with a mark at 20 cc., the dish washed several times with distilled water, the washings being added to the tube, and the volume finally adjusted to the mark. A standard was prepared in an identical tube by treating 2 cc. of a standard potassium dihydrogen phosphate solution (0.2193 gm. per litre = 0.05 mg. per cc.) (15) with the acid and making up to volume. The reagents were then added as follows: 2 cc. of 15 per cent sodium bisulphite containing 0.5 per cent. hydroquinone, followed by 2 cc. of ammonium molybdate according to Briggs (15) - 25 gm. ammonium molybdate C.P. in 300 cc.  $H_2O$  plus 200 cc.  $H_2SO_4$  (3+5). The contents of the tubes were then mixed well, immersed in a boiling water bath for 15 minutes, cooled in running water and made up to 50 cc. volume prior to comparison of the clear blue color in a colorimeter.

## PHOSPHOLIPOID-FAT RELATIONSHIPS IN CREAM

Seven samples of cream of increasing fat content were prepared in the laboratory from the same batch of fresh raw milk by simple centrifugalization in an International type SB centrifuge and analyzed for their total fat and lipid phosphorus contents by the methods outlined. The results are shown in table 2.

TABLE 2  
*Fat and lipid phosphorus contents of creams made by simple centrifugalization of the same raw milk*

CREAM FRACTION	FAT  <i>per cent</i>	LIPID PHOSPHORUS	LIPID PHOSPHORUS IN EXTRACT
		<i>mg. per 100 gms.</i>	<i>mg. per 100 gms.</i>
Raw Milk	3.52	1 13	32.10
1	4 47	1.25	27.96
2	11.16	2 37	21.24
3	19.95	3 53	17.69
4	34.58	5.57	16.10
5	36.52	5 78	15 83
6	40 26	6.16	15.30
7	68.87	6 18	9.97

The values obtained on these samples in general checked those obtained in the preliminary work on the market creams (table 1) to the point of approximately 35 per cent fat content. Above this point, however, the lipid phosphorus content continued to rise with the fat content and the actual point of transition at which the phospholipoids began to diminish as the fat content continued to rise occurred at an obviously higher fat concentration. This might indicate that phospholipoids are destroyed during the pasteurization or aging of cream prior to marketing. Further consideration is given this possibility later in this work and in a subsequent paper.

The wide difference of fat content between samples 6 and 7 rather obscured the actual point of transition at which the phospholipoids begin to decrease as the fat increases continue. It was therefore decided to prepare a set of samples of cream of increasing fat contents according to strict modern dairy practice. Accordingly, sixteen such samples were made in a standard cream separator of the skim-milk screw type using the same batch of fresh raw milk. The fat content was increased by careful regulation of the skim-milk screw. The samples were quickly cooled immediately after their separation and kept at approximately 40° C. until analyzed. The fat and lipid phosphorus contents were then determined in the raw samples and also after pasteurization at 143° F. for 30 minutes. The results are given in table 3.

It is noted that the values indicating the rise in phospholipoid content as the fat content increases check those given in table 2 rather closely and

TABLE 3

*Fat and lipid phosphorus contents of creams prepared in separator from same fresh raw milk before and after pasteurization*

CREAM FRACTION	FAT		LIPOID PHOSPHORUS		LIPOID PHOSPHORUS IN EXTRACT	
	Before pasteurization	After pasteurization	Before pasteurization	After pasteurization	Before pasteurization	After pasteurization
	<i>per cent</i>		<i>mg. per 100 grams</i>		<i>mg. per 100 grams</i>	
Milk	3.12	3.07	0.91	0.94	29.17	30.62
Cream 1	10.46	10.39	1.97	2.10	18.83	20.21
" 2	11.48	11.51	2.25	2.36	19.60	20.50
" 3	13.34	13.39	2.44	2.51	18.30	18.74
" 4	16.00	15.99	2.93	3.01	18.31	18.82
" 5	17.11	17.10	3.22	3.22	18.82	18.83
" 6	19.20	19.15	3.41	3.49	17.76	17.90
" 7	21.39	21.39	3.89	3.61	18.18	16.88
" 8	25.36	25.30	4.33	4.34	17.07	17.15
" 9	37.68	37.81	6.32	6.15	16.77	16.27
" 10	46.77	46.85	7.59	7.78	16.23	16.60
" 11	55.24	55.38	8.49	8.71	15.37	16.72
" 12	58.37	58.48	8.33	8.42	14.27	14.10
" 13	58.69	58.87	8.26	8.45	14.07	14.36
" 14	59.12	59.17	8.14	8.69	13.77	14.09
" 15	59.09	59.15	8.16	8.59	13.80	14.52
" 16		66.63		8.13		12.20

those in table 1 for the creams having fat contents up to 35 per cent. The results shown in tables 2 and 3 are plotted graphically in figures 1 and 2 for the purpose of comparison and visualization of what has occurred.

The transition point (figs. 1 and 2) occurs at 55 to 58 per cent fat content. This seems to be evidence to indicate that rapid coalescence or aggregation of the fat globules has taken place in the cream system at this fat concentration and that there is about to take place a partial reversion of the colloidal system. This can be better understood when it is remembered that lecithin possesses the property of swelling up considerably in aqueous medium and, therefore in milk and cream it probably occupies all of the space between the fat globules forming an aqueous phospholipoid "matrix" for their dispersion. It can accordingly be conceived that the volume of this phospholipoid "matrix" which is distributed uniformly throughout the continuous phase is diminished with the removal of the skim milk, the phospholipoid being itself removed in part.

In figure 2 we note that about 40 per cent of the phospholipoid is removed from the fatty extract as the fat content of the cream reaches approximately 15 to 20 per cent. Then, until the concentration of the fat approaches about 55 per cent, the phospholipoid tends to adhere rather tenaciously to the fat globule. For this reason it is noted that the lipid phos-

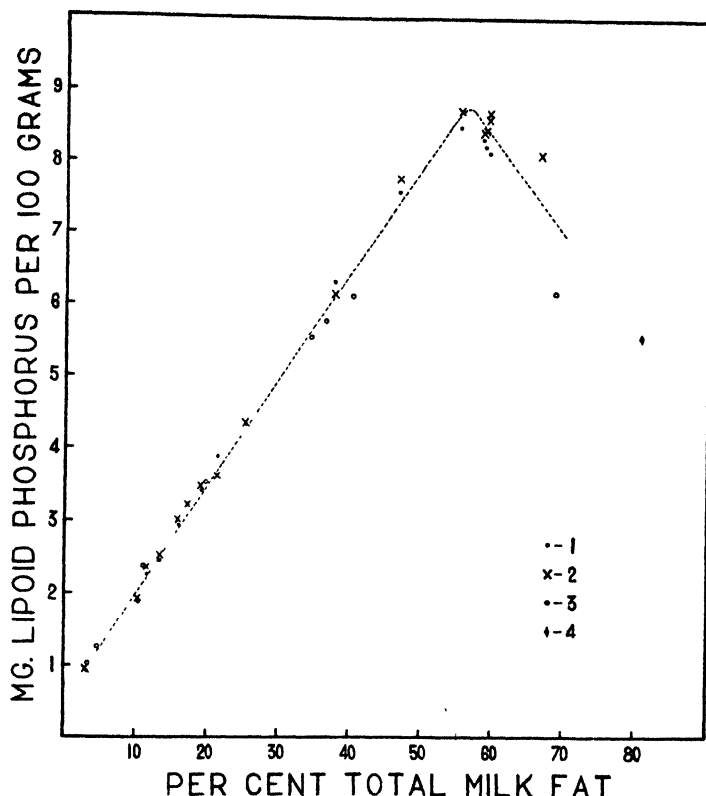


FIG. 1. The variation of phospholipoids with the fat contents of cream: 1—cream produced in cream separator from same batch of raw milk; 2—same cream after pasteurization; 3—cream produced in laboratory by ordinary centrifugalization of same batch of raw milk; 4—butter (Cusick; Smith).

phorus content of the fatty extract remains rather constant in this range, the milk serum carried out with the skim milk taking with it a uniform quantity of the phospholipoid. This obviously concentrates the remaining phospholipoid about the fat globule and results in the formation of a fragile membrane around it, and as more serum is removed the fragility of this membrane is increased until it finally gives way and allows fusion of the unprotected fat mass. The fat becomes continuous and probably occludes a portion of globules surrounded by unbroken membranes. King (16) in a recent work explains butter as a system containing fat in both the continuous and dispersed phases. He maintains that it comprises globules of water and globules of fat surrounded with a stabilizing film in a fatty liquid. This accounts for the presence of phospholipoids in butter. They

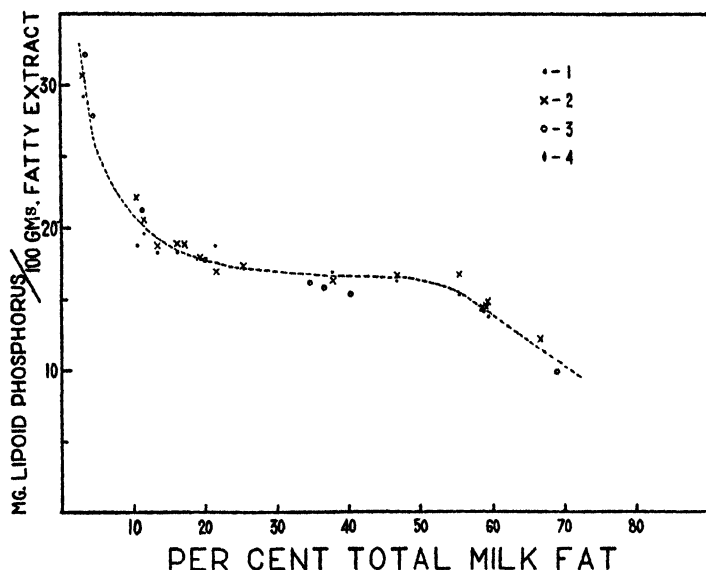


FIG. 2. The variation of phospholipids in the fatty extracts of creams of increasing fat contents: 1—cream produced in cream separator from same batch of raw milk; 2—same cream after pasteurization; 3—cream produced in laboratory by ordinary centrifugalization of same batch of raw milk; 4—butter (Cusick; Smith).

remain due to mechanical occlusion during churning rather than to solution in the fat as is demonstrated by the fact that filtered melted butterfat contains no phosphorus.

It is interesting to note (figures 1 and 2) that Cusick's and Smith's figures for lipid phosphorus in butter fall directly on the line of phospholipid decrease, thus substantiating the facts discussed.<sup>1</sup>

To demonstrate that the original milk phospholipids are divided between the cream and the skim milk prepared from it, samples were taken from a separator run at a local dairy and analyzed as follows:

100 GRAMS MILK—4.24 PER CENT FAT  
containing 1.06 mgs. lipid phosphorus

9.8 grams cream — 41.53 per cent  
fat contains 5.86 mgs./100 gms.  
lipid P or .57 mg.

90.2 grams skim — 0.08 per cent fat  
contains 0.53 mg./100 grams lipid  
P or 0.48 mg.

loss — 0.01 mg. lipid P

<sup>1</sup> Cusick's figure (9) for lipid  $P_2O_5$  in butter is equivalent to 5.56 mg. of phosphorus per 100 grams and Smith (10) gives as averages for 7 samples of sweet butter 5.58 mg. of phosphorus per 100 grams and 80.85 per cent of butter fat.

Another set of samples prepared from raw milk in the laboratory showed the following distribution :

100 GRAMS MILK - 5.35 PER CENT FAT

containing 1.32 mgs. lipid phosphorus

9.9 grams cream - 37.22 per cent  
fat contains 5.44 mgs./100 gms.  
lipoid P or 0.54 mg.

90.1 grams skim - 0.168 per cent  
fat contains 0.82 mg./100 gms.  
lipoid P or 0.74 mg.

loss - 0.04 mg. lipid P

The distribution of lipid phosphorus in creams of increasing fat contents and in the resulting skim milk prepared from 100 gram portions of milk is shown in figure 3.

EFFECTS OF PASTEURIZATION

An attempt was made to verify information given in the literature to the effect that heat causes a diminution in milk phospholipoids (18). The raw milk and cream samples, the results for which are given in table 3 and plotted in figures 1 and 2, were subjected to a pasteurization temperature of 61-62° C. for 30-35 minutes, cooled, and analyzed for fat and lipid phosphorus contents as before. No decrease in phospholipoids was effected by the heating procedure as is evident by the results given in the table and graphs. Further work on the effect of heat on milk phospholipoids is reported in a subsequent paper (17).

CONCLUSIONS

(1) The phospholipoid content of fresh cream increases uniformly with the fat content to a point of approximately 55-58 per cent milk fat when it diminishes with further fat increases.

(2) The phospholipoid content of the fatty extract of fresh cream shows a variable decrease with the increase of the fat content of the cream indicating a variable loss of the original milk phospholipoids.

(3) Approximately 40 per cent of the original milk phospholipoids are removed with the skim-milk in producing cream of from 15 to 20 per cent fat content.

(4) Little further removal of phospholipoids occurs as creams of from 20 to 55 per cent fat content are produced.

(5) Evidence presented indicates the probable occurrence of a reversion of the colloidal system in fresh cream at approximately 55-58 per cent fat content.

(6) No indications were found that heat alone will effect the destruction of phospholipoids in milk products. Further work on the effect of heat on milk phospholipoids is reported in a subsequent paper.

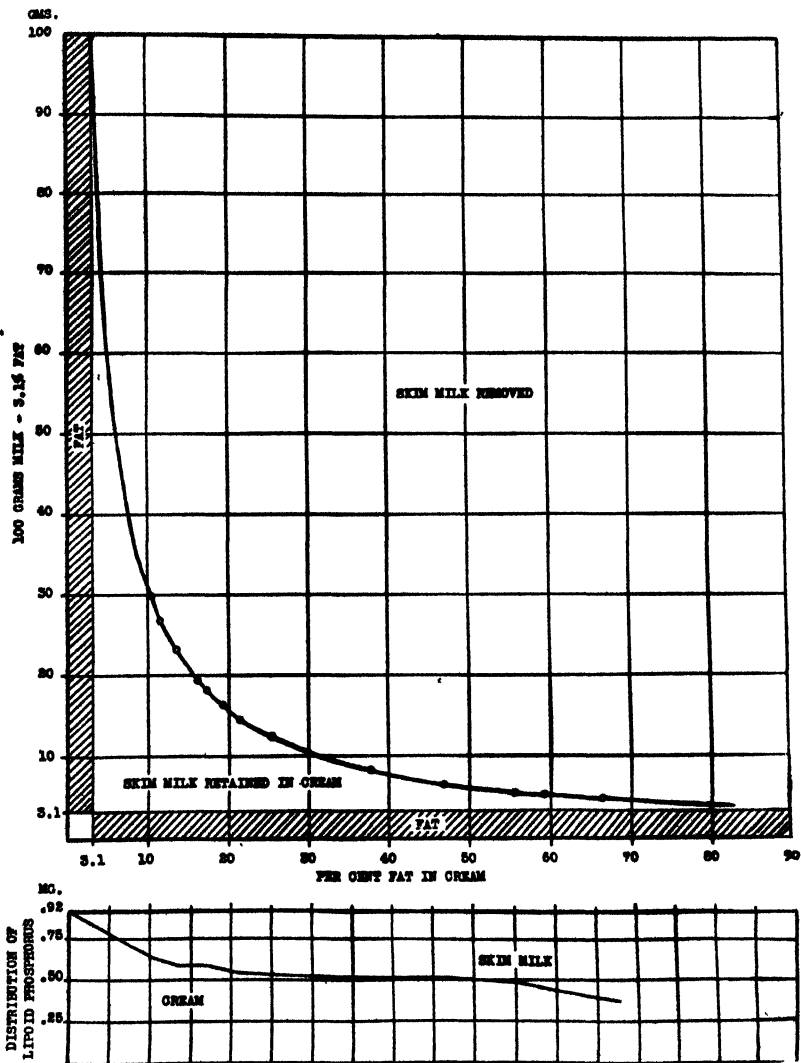


FIG. 3. The distribution of milk lipid phosphorus in cream and resultant skim milk as creams of increasing fat content are produced from 100-gram portions of milk. (Based on table 3.)

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## THE EFFECT OF HEAT ON MILK PHOSPHOLIPOIDS

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The destruction of phospholipoids in milk and milk products during heating is by many considered an accepted and unchallenged truth. Bordas and de Raczkowski (1) in 1903 conducted heating tests on milk and concluded that a considerable portion of the total lecithin present was destroyed at elevated temperatures maintained during 30 minute periods of time. Thus they reported that a sample heated for 30 minutes at 60° C., simulating ordinary pasteurization, lost 14 per cent of its original lecithin content. At 80° C. this was doubled, while no greater losses occurred at 95° C. Another sample heated at 95° C. lost but 12 per cent of its phospholipoid content computed as lecithin while a third sample when held in an autoclave at 105-110° C. for 30 minutes lost 30 per cent of its original lecithin content. It is obvious that no uniform relationship between heating and phospholipoid destruction exists in these reported figures contrary to expectations that such decomposition, if due entirely to heat, would be directly proportional to the degree of heat as well as to the heating time. The results of Bordas and de Raczkowski's work are given in table 1.

TABLE 1

*The destruction of milk phospholipoids by heating over thirty minute periods—  
Bordas and de Raczkowski*

LECITHIN CONTENT	HEATING TEMPERATURE	LOSS
<i>per cent</i>	<i>Heating temperature</i>	<i>per cent</i>
0.0252	60	14
0.0252	80	28
0.0252	95	28
0.0365	95	12
0.0255	105-110	30

In attempted verification of the above, 16 samples of cream of increasing fat content separated from the same batch of fresh raw milk were analyzed for their lipid phosphorus contents, subjected to a pasteurization temperature of 61-62° C. for 30-50 minutes, cooled, and again analyzed as before (2). A drop of diluted formaldehyde had been added to the raw samples for the purpose of retarding bacterial growth subsequent to the removal of portions for the initial analyses. No decrease in phospholipoids was effected by the heating procedure (see table 3 in preceding paper).

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At this point it is interesting to consider Cusick's recomputed values for the lecithin contents of various butters (3). Raw sweet butter showed a decrease in lecithin of from 0.145 per cent to 0.104 per cent in 48 days and no further decrease to 72 days. Pasteurized sweet butter decreased in lecithin content from 0.145 per cent to only 0.136 per cent in 48 days and to 0.101 per cent in 72 days. Raw ripened butter showed a lecithin decrease of from 0.139 per cent to 0.098 per cent in 48 days and to 0.094 per cent in 72 days while pasteurized ripened butter having a lecithin content of 0.090 per cent showed no decrease in 72 days. No initial decrease is shown for sweet butter upon pasteurization while raw ripened butter has 4 per cent less lecithin and pasteurized ripened butter 40 per cent less lecithin. These figures indicate unmistakable bacterial enzymatic decomposition of the lecithin. Apparently the lecithin decomposing enzyme produced during the bacterial ripening process has a rather high optimum temperature as is indicated by the 40 per cent lecithin loss in the pasteurized ripened butter as compared to only a 4 per cent loss in the raw ripened butter.

The possibilities of bacterial enzymatic decomposition of phospholipoids have been studied previously by the writer (4). It was found that there exist certain bacteria which produce powerful extracellular lecithin-splitting enzymes and several strains were successfully isolated from chance inoculated whole mixed eggs. It is probable that bacterial phospholipoid-splitting enzymes having a possible high optimum temperature played a significant rôle in yielding the previously cited results reported by Bordas and de Raczkowski as well as those of Cusick.

To secure further evidence that heat alone is not the factor responsible for the destruction of milk phospholipoids a sample of fresh raw milk produced under very carefully controlled sanitary conditions and having an initial bacterial plate count of 3,500 per cc. was obtained, analyzed for lipid phosphorus content, and 100 cc. portions subjected to the action of heat and known phospholipoid-splitting bacteria. The portions were then again analyzed to ascertain any loss in phospholipoid content. The treatments and results obtained are given in table 2.

It is evident that heat causes no change in the phospholipoid content of the milk. Higher autoclaving temperatures than those used by the previous investigators were applied to portions 3 and 4. Very evident caramelization occurred in portion 4. The total time this portion remained at a temperature above 100° C. was well over an hour, yet no diminution of phospholipoids occurred. The slight differences in the results can be regarded to be well within experimental error.

It might be well to mention at this point that continued heating at 100° C. of the extracted fatty residues of both egg and milk products after constant weight is obtained yields no diminution in lipid phosphorus.

The bacteria present in the milk upon its receipt were obviously not of the lecithin-splitting type. This is doubtlessly due to the carefully con-

TABLE 2  
*The effect of heat and lecithin-splitting bacteria on milk phospholipoids.*

PORTION AND TREATMENT	TOTAL FAT	LIPOID PHOSPHORUS	LIPOID PHOSPHORUS IN EXTRACT
	<i>per cent</i>	<i>mg./100 grams</i>	<i>mg./100 grams</i>
1. Raw fresh milk as received	3.98	1.20	30.15
2. Pasteurized portion—kept at 60–65° C. for 30 minutes	4.05	1.18	29.14
3. Autoclaved portion—kept at 120–125° C. for 15 minutes	4.07	1.22	30.00
4. Autoclaved portion—kept at 120–125° C. for 30 minutes	4.03	1.23	30.52
5. Raw portion—incubated at 37° C. for 48 hours	3.90	1.21	31.02
6. Raw milk plus 1% 48 hr. broth suspension of lecithin-splitting bacteria incubated at 37° C. for 24 hours	3.99	0.54	13.53
7. Portion No. 6—incubated at 37° C. for 48 hours	3.98	0.43	10.81

trolled sanitary conditions under which the milk was produced. A gassy curd was produced after 48 hours at 37° C. but there was no decrease in the phospholipoid content.

Portion 6 and 7 were inoculated with a 48 hour broth culture of the tentatively named *Bacillus lecitoris*, *nov. sp.* which had been isolated from decomposing mixed eggs into which it found its way by chance inoculation. This microorganism has been found to produce an extra-cellular enzyme capable of destroying completely the phospholipoids present in eggs (4). In milk it was found to destroy 65 per cent of the phospholipoids in 48 hours at 37° C. At least three other organisms capable of producing lecithin-decomposition have been isolated in this laboratory but their identification is as yet incomplete.

The experimental technic and methods employed in this work were identical to those used in the previously reported study of the phospholipoid distribution in cream (2).

#### SUMMARY

No evidence that heat alone causes the destruction of lecithin and its allied phospholipoids in dairy products was obtained. This is in contradiction to previously reported results obtained without the consideration of the

probable presence of phospholipoid decomposing bacterial enzymes. It is shown that certain bacteria are capable of producing enzymes which will effect the destruction of milk phospholipoids.

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## A WATERING DEVICE FOR EXPERIMENTAL WORK

A. D. PRATT AND H. A. EDGE

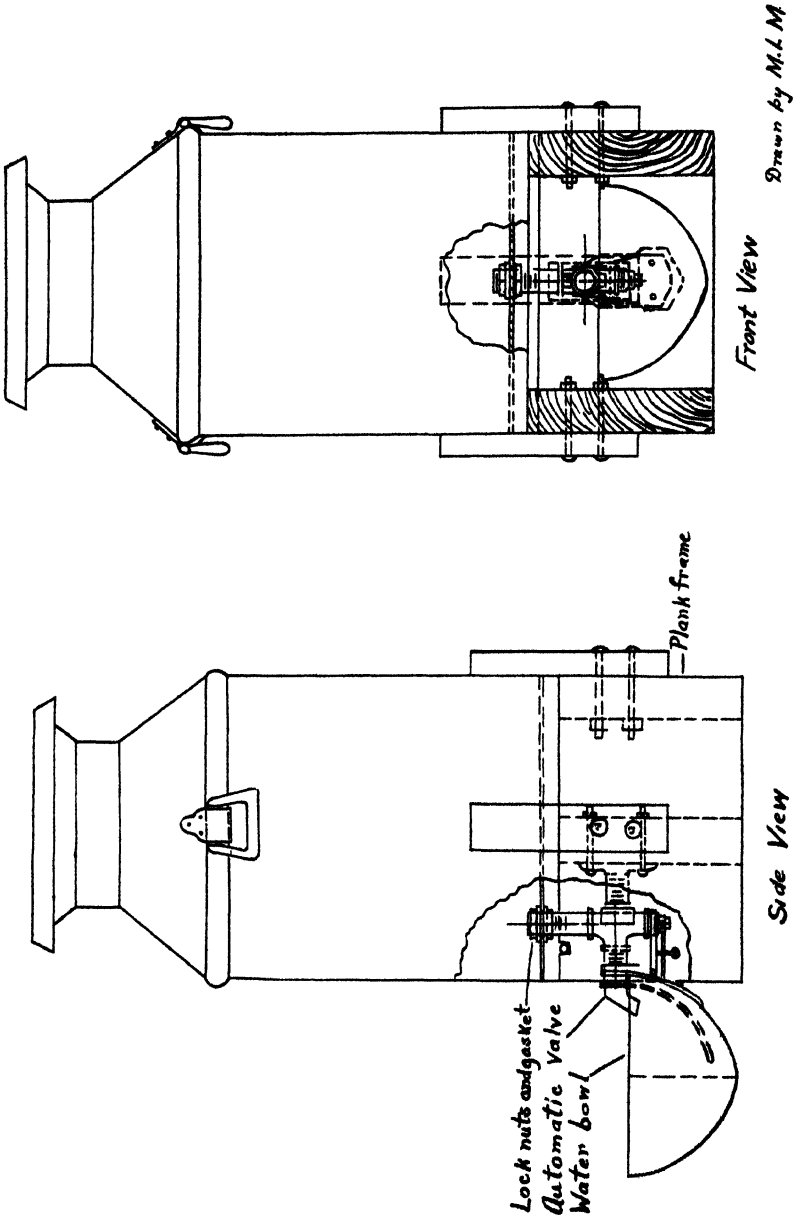
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Some types of experimental work with dairy cattle require that the water intake be measured. When adequate numbers of experimental animals are used, the cost of water meters to individual stalls is often prohibitive. The accompanying cut shows our solution of this problem.

A used ten gallon milk can was mounted on a plank frame which was high enough to allow attachment of a standard Star drinking cup. The cup was attached to the can by means of standard pipe and fittings. The attachment to the can was by means of lock nuts and a rubber gasket.

Water was weighed or measured into the can and unused portions weighed back or the amount required to refill the can was recorded. Very little difficulty was experienced with leakage or spillage, and the device, although inexpensive, was altogether satisfactory for our purpose.

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# A STUDY OF THE RELATION OF MATERIALS ADSORBED ON THE FAT GLOBULES TO THE RICHNESS OF FLAVOR OF MILK AND CERTAIN MILK PRODUCTS\*

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The peculiarly rich flavor of buttermilk was noted by Sammis (8) in 1914 and again (9) in 1920. The authors of this paper have observed repeatedly that the buttermilk resulting from the churning of sweet cream possesses a flavor richness that is about equivalent to that of normal whole milk, although the butterfat content of whole milk usually is about eight times that of buttermilk. It seemed likely, inasmuch as buttermilk is relatively richer in phospholipids than whole milk or skimmilk, that these compounds might be responsible in some way for a large portion of the flavor richness observed in buttermilk. It also seemed obvious that, if phospholipids contribute to the rich flavor of buttermilk, they also should be responsible for a portion of the richness of flavor in milk products which contain them, whereas milk products so treated as to remove a portion of the phospholipids might be expected to have lost some of their flavor richness.

An excellent summary of the findings of various workers relative to the phospholipid content of various milk products is given by Holm, Wright and Deysher (5). These authors also have determined the phospholipid content of milk and the products manufactured from it. Their work indicates that the greater portion of the phospholipids of normal milk occurs in the plasma and not adsorbed on the fat globules, as over 70 per cent of the phospholipids remained in the skimmilk after separation. However, their results confirmed the suggestion of Thurston and Petersen (11) that the phospholipid content of buttermilk increases as the butterfat content of the cream churned increases. This is shown by the excerpts from their data given in table 1.

Palmer and Samuelson (6) and Palmer and Wiese (7) have shown that phospholipids form a part of the adsorbed film on the fat globules in normal milk, and that these substances are recovered in large proportions in the buttermilk after churning cream washed eight times by dilution with water and reseparation. These findings explain why the phospholipid content of buttermilk is higher than that of whole or skimmilk and indicate that an increase in phospholipid content of buttermilk should be expected when an

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\*\* Most of the data presented herein were included in a thesis presented by J. L. Barnhart in partial fulfillment of the requirements for the degree of Master of Science, West Virginia University.

TABLE 1  
*The phospholipid content of buttermilk from cream of varying richness  
 (After Holm, Wright and Deysher (5))*

FROM MILK NO.	BUTTERFAT CON- OF CREAM	PHOSPHOLIPID CONTENT OF BUTTERMILK
		<i>Per cent</i>
1	27.0	0.284
2	34.0	0.318
3	42.0	0.382

increase in the butterfat content of the cream from which it is churned occurs because of the concentration of an increased amount of "hulls" in a reduced quantity of plasma.

At the West Virginia Station a method has been used of estimating the phospholipid content of dairy products based on extraction of the Roese-Gottlieb fatty residue with three ten-ml. portions of acetone, evaporating the acetone, weighing the residue considered to be butterfat, and determining the phospholipids by difference. This method admittedly yields only approximations, as it has been shown (1) (2) (5) that ether extraction as carried out in the Roese-Gottlieb procedure fails to remove all the phospholipids from the plasma. However, when applied to buttermilk, this method yields differences which are in line with the theory, and has been shown (10) to be very accurate for prepared fat-phospholipid mixtures. Table 2 shows these results. The data indicate, as do those of Holm *et al.* (5), that the phospholipid content of buttermilk increases as the fat content of the cream churned increases.

In view of the foregoing information the production of buttermilk from very rich cream was selected as a means of obtaining a milk product relatively rich in phospholipids. For purposes of flavor comparisons, milk products of medium and low phospholipid content were desired also. Whole milk standardized with skim milk to the percentage butterfat content of the buttermilk served as the product of medium phospholipid content. The preparation of a milk product of low phospholipid content was accomplished by emulsifying washed butterfat in skim milk by means of a homogenizer at pressures such that the fat globules were approximately of the same average size as those in the original whole milk as determined by comparison under the microscope.

The washed butterfat was prepared by repeatedly diluting cream and separating it in a de Laval No. 17 separator. About two quarts of rich cream were diluted to 10 gallons with water at approximately 100° F., separated, rediluted, and separated until each cream had been washed 14 times or more. Near the end of this procedure, usually at the completion of the twelfth washing, a noticeable oiling off of the butterfat occurred which indi-

cated that the "hull" substances had been largely removed. Further washing appeared to cause a complete oiling off of the butterfat.

Three different butterfats prepared in this way were analyzed for organic phosphorus. For this determination the fats were fused in a fusion mixture composed of two parts sodium carbonate and one part potassium nitrate. After fusion the mixture was dissolved in hot water and hydrochloric acid, and phosphorus was determined by the colorimetric method of Fiske and Subbarow (4). No trace of phosphorus was found in any of the samples, thus indicating that the washing removed all or practically all of the phospholipids adsorbed on the fat globules in the normal milk. In each series the buttermilk and the milk remade by emulsification of washed butterfat in skimmilk were prepared from the same milk that was used as the product of medium phospholipid content. Five such series were prepared and judged.

Regular analyses of each series judged for flavor richness were not made. One series was analyzed, however, to make certain that the expected differences in phospholipid content actually occurred. In this series the cream used was considerably lower in butterfat content than in the other series studied. The phospholipid content was determined as follows: A macro-Roese-Gottlieb extract was prepared according to the method of Bird and Sands (1). The phosphorus content of the fatty extract obtained in this manner was determined by the method described above for the determination of phosphorus in washed butterfat, and the percentage of distearyl

TABLE 2

*The quantities of ether-alcohol soluble but acetone insoluble substances in buttermilks from creams of varying richness*

MILK	CREAM	FAT CONTENT OF CREAM (BABCOCK)	ETHER-ALCOHOL SOLUBLE, ACETONE INSOLUBLE SUBSTANCES IN BUTTERMILK
		<i>Per cent</i>	<i>Per cent*</i>
A	1	18	0.2145
	2	43	0.2982
	3	71	0.4874
B	1	17	0.2356
	2	37	0.4578
	3	64	0.5580
C	1	12	0.1652
	2	26	0.1966
	3	66	0.3863
D	1	24	0.2023
	2	39	0.2609
E	1	20	0.2609
	3	66	0.3460

\* Average of duplicate determinations.

TABLE 3  
*The phospholipid contents of a series of three milk products prepared for  
 flavor comparisons*

MILK PRODUCT	PHOSPHOLIPID CONTENT	
	Duplicates	Average
	<i>Per cent</i>	<i>Per cent</i>
Buttermilk from cream containing 35% butterfat	0.0911 0.0885	0.0898
Normal whole milk	0.0287 0.0291	0.0289
Milk prepared by emulsifying washed butterfat in skimmilk	0.0168 0.0164	0.0166

TABLE 4  
*Comparison of the richness of flavor of buttermilk and skimmilk standardized to the  
 same fat content (Babcock test) with normal cream and with washed butterfat*

ORDER OF FLAVOR RICHNESS	BUTTERMILK*	SKIMMILK STANDARDIZED WITH NORMAL CREAM	SKIMMILK STANDARDIZED WITH WASHED BUTTERFAT
First	20	3	5
Second	5	19	4
Third	3	6	19

\* Two of the buttermilks gave a noticeable oxidized flavor.

lecithin was calculated from the quantity of phosphorus found. The results are shown in table 3.

It will be observed that these results are lower throughout than those of Bird and Sands and still lower than those of Holm *et al* (5). This probably was due to the method used rather than to differences in phospholipid contents of the products studied by the different workers. However, the results shown in table 3 indicate the important point concerned in this study, *viz.*, that the samples prepared were of relatively high, medium, and low phospholipid content, respectively. The phospholipids found in the sample made up from washed butterfat and skimmilk are undoubtedly from the skimmilk inasmuch as the analysis of the washed butterfat as reported above showed no determinable trace of phospholipids.

Each series of milks was judged as soon as it was prepared. The samples were judged by a group of men familiar with dairy products who were not informed of the identity of the samples. They were selected on the basis of their ability to detect differences in flavor richness in standardized milk samples varying in butterfat content from 1 to 4 per cent, with regular differences of 0.5 per cent between samples. Seven were able to

place these samples in their exact order of richness or nearly so and were selected as judges. There were four of these men who judged the experimental milks regularly while one or more of the others usually was available.

The results of judging the five series of milks are shown in table 4. The data show a striking relationship between the method of preparing the samples and their flavor richness. Inasmuch as the samples compared within each trial were standardized to the same butterfat content, on the basis of the Babcock test, it is reasonable to assume that the difference in richness of flavor observed was due to variations in the amounts of "hull substance" present. Because phospholipids are glycerides of a molecular configuration similar to butterfat, and because the order of flavor richness of the samples is directly related to the amount of phospholipids expected to be present due to the methods of preparing the samples, it is indicated that phospholipids contribute to the rich flavor of milk.

In order to make a more direct determination of the effect of lecithin on the flavor of milk, milk lecithin was prepared and dispersed both in skim-milk and in synthetic milk. The milk lecithin was prepared by purification of the alcohol extract of dry buttermilk. Two shotgun cans were partly filled with 98 per cent ethyl alcohol, and 25 pounds of fresh dry buttermilk were stirred into each. The mixtures were allowed to stand for three days, being frequently stirred. Occasional additions of alcohol were made to replace that which had evaporated. At the end of this period the alcohol was removed by means of a hydraulic press operated at a pressure of approximately two tons per square inch. The alcohol solution then was placed in wide, enameled trays and the alcohol evaporated at room temperature with the aid of an electric fan. The residue was taken up in a small volume of ether and, without filtering, precipitated with acetone and pounded together with a glass rod. The acetone-ether mixture was decanted and the precipitate dissolved in ether, precipitated, and treated as before. This procedure was repeated three times to remove all but traces of acetone-soluble compounds. The precipitate then was emulsified in a large excess of water to which a small amount of sodium chloride had been added, after which it was reprecipitated by the addition of a quantity of acetone equal to one-half of the volume of the emulsion. The precipitate then was removed, reemulsified, and treated in the same manner. This was repeated three times. The final precipitate was dried by treating with acetone, as much acetone as possible pressed out, and the whole taken up in ether. The ether solution was allowed to stand in tall cylinders until the white precipitate, sphingomyelin, settled, after which the ether solution was decanted and treated with an excess of acetone. The resulting precipitate again was taken up in ether and the process repeated. The substance then was dissolved in alcohol and any precipitate allowed to settle out, after which the alcohol was evaporated. The residue then was taken up in ether

and precipitated with acetone. Finally it was treated with several changes of acetone and dried in a desiccator, in vacuum over sulphuric acid. The substance thus purified is believed to be a mixture of lecithin and cephalin. However, it possesses all the properties of the substances generally referred to as lecithin.

Synthetic milk was prepared according to the method of Clark (3) except that the casein used was prepared according to the method of Van Slyke and Bosworth (12). To one sample of synthetic milk was added .5 per cent of washed butterfat, prepared as previously described, to a second sample .5 per cent of lecithin was added, and to a third an amount of gum arabic equal to that used to emulsify the washed butterfat in sample one. A similar set was prepared using skimmilk in place of synthetic milk. The judges were unanimous in placing the lecithin samples first, the washed butterfat samples second, and the gum arabic samples last on the basis of rich flavor. They also observed that the samples containing lecithin were distinctly oxidized in flavor and the sample prepared from skimmilk seemed to be identical in flavor to the buttermilk samples in which an oxidized flavor was noted previously.

#### CONCLUSIONS

That the phospholipids of milk contribute to the richness of flavor in milk products is indicated by the results herein reported.

Buttermilk from sweet cream has a rich flavor probably because of the presence of relatively high percentages of phospholipids left in the buttermilk as the result of their disengagement from the fat globules during churning.

#### ACKNOWLEDGMENT

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# THE VOGT METHOD OF MANUFACTURING FLAKE BUTTERMILK

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The Vogt method of making flake buttermilk was discovered by Gerald Vogt, Assistant Superintendent of the Cohocton plant of the Wetmiller Dairy and Farm Products Company, Inc., Cohocton, New York. It is a simple method of forming flakes or granules of butter in buttermilk that can be employed on either a small or a large scale. It consists of spraying melted butter into cold cultured buttermilk during constant agitation.

## DETAILS OF MANUFACTURE

The first step in the manufacture of this product is to make a superior cultured buttermilk. In the manufacture of cultured buttermilk, it is desirable to select good skimmilk. Then, usually, salt should be put into the skimmilk at the rate of two to four ounces per ten gallons of skimmilk. The purpose of adding the salt is to improve the flavor and to make a smoother, or less lumpy buttermilk. At this point the skimmilk should be pasteurized by heating to a temperature of approximately 190° F. for 30 minutes, after which it should be cooled to a holding temperature of about 70° F., when it must be inoculated with a good starter. The incubation or ripening period will require about 12 to 15 hours. An acidity of approximately .75 per cent total acidity expressed as lactic acid, ordinarily is satisfactory.

This curdled skimmilk now should be cooled to approximately a storage temperature of about 40° F. during which time sufficient agitation must be applied to assure a smooth, or lumpless body. It can then be sold as cultured buttermilk or used in making flake buttermilk by the Vogt method.

For the Vogt method, a sufficient amount of good butter should be melted to supply all, or a part, of the milkfat up to 1 per cent. If the buttermilk is to contain 1 per cent milkfat, some of it could well be added in the form of homogenized cream. The milkfat that is to be supplied in the form of flakes should be heated in the sprayer to 120° F., at which time the butter color should be put in. Usually about five to ten times as much color should be used as in the manufacture of butter, for it is essential that the flakes be easily seen. On the other hand, they should not be too highly colored. Mr. Vogt advises that water should be added to the butter, making a mixture of water and milkfat of about one-third water to two-thirds milkfat.

A good sprayer is essential. The one used in this experiment was

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Brown's Auto-spray which is commonly used for spraying fly-killing mixtures, disinfectants, and the like. Other sprayers may do just as well. At this time in the process the water-milkfat mixture should be heated in the sprayer to 180° F. for several minutes, in order to kill the microorganisms in both the mixture and the sprayer. Then the contents of the sprayer should be cooled to about 120° F., when the milkfat may be sprayed directly on to the surface of the cold-cultured buttermilk while it is being constantly agitated. During this stage of the process the sprayer should be shaken often, so that the water and milkfat will be maintained in a reasonably good emulsion. If it is desirable to make a finished product containing one per cent milkfat, or any other definite amount, the loaded sprayer should be weighed and then as the load is reduced in weight, additional weighings should be made.

#### OBSERVATIONS

The flakes in the buttermilk that have been made by the Vogt method are flat and somewhat ragged in general appearance, and they vary considerably in size. Uniform spraying and agitation are the main factors in controlling the size of the flakes. The particles of butter that are formed in the churning method are more uniform in size, and they are round.

The sanitary feature of the Vogt method is important. The sprayer which is made of metal is readily cleaned and scalded. The wooden churn, on the other hand, is most difficult to keep sanitary, particularly when it is not used daily.

The Vogt-method buttermilk is easily and economically made for the cultured buttermilk from which it is manufactured can be stored, and within five minutes it can be transformed to flake buttermilk. Also, if on account of age or other reason, the original-stock supply goes bad, the milkfat is not lost, for it may be added only when the flake-buttermilk order has been received.

A comparative body and flavor study was made of (a) churned-cream buttermilk, (b) churned-flake buttermilk, and (c) Vogt-method buttermilk. Four sets of five samples each were run in which a sample of each set was examined on five consecutive days.

The body of the churned-cream buttermilk was free from lumps and poured like sweet milk. The other two buttermilks possessed rather heavy bodies with casein particles sufficiently large to appear lumpy. The flavor of the churned-cream buttermilk was preferred by persons accustomed to the "old fashioned" buttermilk in which there is a definite flavor of the butter. The other two buttermilks contained a fatty flavor that approached that of butter. The flavor of all three types of buttermilk was maintained splendidly for five days.

## American Dairy Science Association Announcements

ANNUAL MEETING AMERICAN DAIRY SCIENCE ASSOCIATION,  
UNIVERSITY OF MINNESOTA, UNIVERSITY  
FARM, ST. PAUL, JUNE 25 TO 27, 1935

### GENERAL INFORMATION

The meeting will open officially on Tuesday, June 25th. Registration will begin on Monday, June 24th, and a conference on judging dairy products will be held during the afternoon of that day. There will be no conference on judging dairy cattle this year. Rooms will be available in the College of Agriculture dormitories at 75¢ per person per night. Further details will be published in the succeeding issues of the JOURNAL OF DAIRY SCIENCE.

### CALL FOR PAPERS AND ABSTRACTS

*This is the first but not final official call for papers for the scientific sessions of the Association. Individual notices will be sent to members or to Department Heads. It is anticipated that scientific programs definitely will be conducted in the subject matter fields of production, manufacturing and extension. Interest is being manifested in possible programs regarding dairy engineering and regarding teaching problems and methods, as announced in the November 1934, and January 1935, issues of this Journal. Tentative plans are under way for a general scientific session to be held jointly with other agricultural science associations that will be in session at the University of Minnesota during the same week. It is possible that this meeting will be called a meeting of Section O of the A. A. A. S. that will also hold its summer meeting at the University of Minnesota during the week of June 24th.*

Members are invited to send titles of papers to the program committee. Non-members are permitted to read papers if a member of the American Dairy Science Association is a co-author. All papers must represent original work not previously published.

*Titles of papers should be accompanied by or followed by abstracts both of which must be in the hands of the chairman of the program committee by May 1st. Authors are invited to indicate the section before which they desire to present their paper.*

The members of the program committee are H. A. Ruehe, J. M. Sherman and L. S. Palmer, chairman. The latter may be addressed at Biochemistry Building, University Farm, St. Paul, Minnesota.

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*The American Dairy Science Association* was organized to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

Membership shall consist of two kinds: (1) active, (2) associate.

The qualifications for membership in the two classes are as follows: (a) Any person is eligible to active membership who is formally announced by an Agricultural College, or Experiment Station, or by the Bureau of Dairying of the United States Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or (b) anyone filling a position of responsibility connected with the dairy industry and who has had a college or University training in technical science, or anyone filling a responsible position in the industry of a professional character requiring a technical knowledge of dairying of a high order.

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The dues are \$5.00 a year for active membership. Correspondence regarding membership and dues should be addressed to R. R. Graves, United States Department of Agriculture, Washington, D. C.

# JOURNAL OF DAIRY SCIENCE

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## THE RELATION OF THE FAT CONTENT OF MILK TO THE PASSAGE OF THE MILK CURD FROM THE STOMACH OF THE CALF\*

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When the calf has reached the age of three weeks or more, it is the practice of most dairymen to replace the whole milk with skim milk, supplemented by grain and hay. The removal of the fat from the milk narrows the nutritive ratio of the milk from 1:3.9 to 1:1.5. The addition of grain and roughage tends to widen this ratio again. Though the calf's stomach is especially designed to handle large amounts of fiber, there is still some question as to the age at which it is most practical to begin replacing a fiber-free energy food, like fat, with large amounts of grain and roughage.

In addition to the physiological effect of bulk the omission of fat may be of importance in other ways not generally recognized. For instance, the calf is very susceptible to bowel disturbances during the first few weeks of its life. Favorite remedies at this time have been to dilute the milk with lime water, add barley water, or, boil the milk. All of these modifications of milk are designed to make it more digestible in the sense that a more friable, more easily liquefied curd is formed in the stomach. Other nutritive changes may of course accompany these modifications. On the other hand it is generally recognized (3), (4), (7), that fat tends to inhibit the rate of evacuation of the stomach. Alley, MacKenzie and Webster (1) are of the opinion that fat diminishes the peptic power of the gastric juice. The question immediately arises, does the amount of fat commonly found in milk play a favorable or unfavorable part during these periods of indisposition? Is it desirable or undesirable to speed up the rate of evacuation of the stomach at this time and what part does the fat in whole milk play in controlling the stomach processes?

The nutritive value of the fat is unquestioned. Not only does fat serve as a source of energy and as a carrier of the fat soluble vitamins but possibly as the source of certain fatty acids essential to life. However, the

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economy of whole milk as a food source makes a knowledge of the body reserves of these elements important.

#### EXPERIMENTAL

Two trials in consecutive years were run in obtaining the data to be reported. In both trials, calves with rumen fistulas were used. The fistulas were usually made when the calves were about two weeks old. The test meal was placed in a liter bottle and allowed to flow directly into the abomasum through a one-quarter inch rubber tube. By running the tube through the fistula directly into the stomach the effects of dilution by saliva or fluid in the rumen was avoided. No grain or hay was fed while the trials were in progress. At varied intervals, however, the muzzles were removed and the calves allowed to eat *ad libitum*. Before a trial was started the rumen and reticulum were washed out. Sufficient time was then allowed to elapse until no food could be found in the omasum or abomasum. A mineral mixture of salt, steamed bone meal, iron oxide, copper sulfate and potassium iodide was fed, supplemented by cod liver oil.

The time required for liquefaction and evacuation of the stomach was determined by palpating the curd mass through the walls of the rumen and abomasum. In calves, the abdomen lies almost directly beneath the rumen when the latter is empty. As the walls of both stomachs are relatively soft and elastic, any object the size of a pea or larger can be readily located in the abomasum by palpating the floor of the rumen. In fact the authors feel that the exact time at which all food leaves the stomach can be more readily determined in this way than by either Röntgen ray photographs or by acidity curves. The use of acidity curves to supplement the palpation method would of course, be desirable. However, no method probably disturbs the normal physiological processes less than palpation if the effect of barium sulfate in the meal is considered in the taking of Röntgen ray photographs. In fact, after some experience the hand can be inserted into the rumen without the calf being scarcely more than conscious of the act.

For the first few trials the abomasum was palpated every hour after feeding. Near the end of the trial the stomach was palpated every fifteen to twenty minutes to determine the exact end point. When the first few trials were completed and the length of the digestive period for the respective calves determined, then only the palpations at the end of the period were required.

Digestion was considered sufficiently complete when only small amounts of curd (5 to 10 grams) could be located by palpating the abomasum. As soon as this stage was reached the fingers were inserted into the abomasum and the remaining curd removed and weighed. These curds served as a check on the end point of digestion.

In the first series of trials (5) the milk from two Holstein cows was

mixed and used. All milk was separated, standardized to the desired fat content and then viscolized to prevent the fat from separating out. The skim milk was also viscolized. The skim milk trials were conducted first, followed by those with three and six per cent milk respectively.

In the second series of trials an attempt was made to avoid any effect of advancing age by alternating the skim milk trials with those comparing three per cent and six per cent milk. The average time for the evacuation of the stomach with each type of test meal was then computed for each calf. The calves were not fed for 24 hours previous to being given the test meal. Eight hours before the test meal was given the abomasum was examined for any food present and all curds removed manually. Never more than two trials were carried out in any one week in order that the calves might regain any lost weight as a result of the previous trial. The milk used throughout the second series of trials came from different cows from those used in the first series but the same milk was used in each trial. However, the curd tension of this milk was very similar to that used the previous year. In these latter trials whole milk was used instead of milk standardized to three per cent fat. The milk actually tested about 2.8 per cent. The milk standardized for the high fat trials actually tested 5.8 per cent.

Two other slight differences between the two series should be mentioned. In the first series, no attempt was made to control the rate of flow of the milk into the stomach nor was the temperature of the milk closely controlled except to be sure that it was warm. In the second series of trials the rate of flow of the milk was adjusted so that approximately two minutes were required for the test meal, one liter, to flow into the stomach. This was done in an attempt to simulate conditions occurring when the calf sucks the cow. The temperature of the milk was also closely controlled between 35° and 37° C.

#### RESULTS

From table 1 it will be seen that in each case one liter of skim milk required longer to leave the stomach than one liter of milk containing three per cent fat. Also, one liter of three per cent milk required longer to leave the stomach than the same amount of milk containing six per cent fat. The difference in time between the trials with skim milk and those with milk containing three per cent fat is greater, however, than between the trials with milk containing three and six per cent fat.

In table 2 the same general trend is observed though the results are less uniform. No figures are given for the amount of curd removed as an arbitrary value was chosen by which the time for complete evacuation of the stomach was calculated. Curds weighing five grams or less were assumed to "digest" at the rate of five grams per hour. Curds weighing six to ten grams were assumed to "digest" at the rate of six grams per hour. No dif-

TABLE 1  
*The relation of the fat content of milk to the passage of the milk curd from the stomach of the calf*  
 Series 1

KIND OF MILK	TRIAL NO.	CALF 47J			CALF 47K			CALF 47L			CALF 47M			AVERAGE OF ALL TRIALS
		Evac. time in min.*	Wt. of curd in grams**	Evac. time in min.	Wt. of curd in grams	Evac. time in min.	Wt. of curd in grams	Evac. time in min.	Wt. of curd in grams	Evac. time in min.	Wt. of curd in grams	Evac. time in min.	Wt. of curd in grams	
Skim milk	1	340	2.6	345	2.3	315	0.3	315	0.3	425	4.5			Digestion period 325 min.
	2	335	16.1	320	8.3	300	4.9	300	4.9	345	2.9			
	3	310	7.4	320	1.3	300	1.8	270	6.1	355	2.6			
	4	310	6.2	305	6.5									
	5	305	3.1	305	0.4									
	6	305	1.4	285	3.6									Weight of curd 3.9 grams
Average		317	6.1	313	3.7	296	3.3	296	3.3	375	3.3			Digestion period 281 min.
3 per cent milk	1	275	5.7	290	6.1	350	1.9	350	1.9	315	9.5			Weight of curd 3.3 grams
	2	265	1.1	290	0.6	265	2.7							
	3	255	2.4	270	3.2									
	4	235	1.4	260	5.0	245	0.3	245	0.3	310	1.6			
	5	235	1.1	240	2.5	215	2.3	215	2.3	305	3.6			
	6	225	3.4	220	1.9									Weight of curd 3.3 grams
Average		248	2.5	262	3.2	269	1.8	269	1.8	310	4.9			Digestion period 239 min.
6 per cent milk	1	275	0.9	285	3.9	285	1.3	285	1.3	260	2.5			Weight of curd 2.0 grams
	2	265	1.4	285	2.1	265	0.4							
	3	230	2.1	250	3.6	245	2.3	245	2.3	240	1.3			
	4	225	1.9	235	3.2	230	2.1							
	5	220	1.1	225	0.5									
	6	215	3.5	215	1.4									
Average		238	1.8	240	2.5	236	1.5	236	1.5	248	2.4			

\* Length of time from the ingestion of the milk to the removal of any unliquefied curd.

\*\* Dry weight of curd still remaining in the stomach at the end of the test period.

TABLE 2

*The relation of the fat content of the milk to the passage of the milk curd from the stomach of the calf*

*Series 2*

CALF NO.	NO. OF TRIALS	KIND OF TREATMENT	TOTAL TIME FOR LIQUEFACTION OF CURD IN STOMACH	
47-R	9	Skim milk	16 hrs.	20 min.
"	14	3 per cent milk	12 "	22 "
"	4	6 per cent milk	10 "	39 "
47-X	7	Skim milk	16 hrs.	26 min.
"	9	3 per cent milk	12 "	57 "
"	4	6 per cent milk	12 "	3 "
47-A-3	4	Skim milk	19 hrs.	13 min.
"	5	3 per cent milk	17 "	21 "
"	6	6 per cent milk	15 "	45 "
47-A-4	6	Skim milk	17 hrs.	41 min.
"	6	3 per cent milk	15 "	35 "
"	5	6 per cent milk	13 "	15 "
	Total Number Trials			
Average of 4 calves	26	Skim milk	17 hrs.	25 min.
	34	3 per cent milk	14 "	34 "
	19	6 per cent milk	12 "	56 "

ference was made between curds from skim milk, milk containing three or six per cent fat.

#### DISCUSSION

In this study no measure was made of the stage of proteolysis reached by the milk curd but simply determinations of the rates at which the protein curds were liquefied and left the stomach. Several physiological disturbances, however, may have influenced liquefaction. Even the same stomach rarely digested aliquot samples of milk in the same length of time, while the variation in digestion time between the different calves was even greater.

Though there is some indication that the curd formed from skim milk leaves the stomach more slowly than when either three or six per cent fat has been added, the difference is not highly significant due to the marked variation in evacuation time between trials with the same kind of milk. Changes in barn temperature, appetite and the changing state of health of the animals made it impossible to reduce these daily fluctuations to the point desired. In fact, the length of the digestive period was a good index to the general physical condition of the calves.

No definite explanation can be given to show why the calves in one series always "digested" the test meals more rapidly than those of the other series. The viscolization of the milk may have been responsible for the difference.

Quigley, Zettleman and Ivy (6) state that the inhibitory action of fats on gastric secretion and gastric motility is due to a humoral effect set up by the fats while in contact with the mucosa of the small intestine. Alley, MacKenzie and Webster (1) are of the same opinion except that they lay more stress upon the nervous phase of gastric secretion. Apparently the calf does not have such a mechanism or else the percentage of fat in the milk is not sufficient to give a pronounced inhibitory effect. The last explanation would seem much more logical. In fact, Boggess, Bessie and Ivy (3) found that limited amounts of fat did not inhibit the passage of food from the stomach. Bergeim and coworkers (2) found that skim milk required longer to liquefy and escape from the stomach than did milk with four per cent fat. In our own experiments the curd which was formed from skim milk became semi-opalescent in color and tenacious, almost like rubber, in texture while the curd from milk containing six per cent fat was very friable, like cottage cheese in consistency. The curd from milk containing three to six per cent of fat produces a much softer curd in the stomach of the calf than fat-free milk while this amount of fat is sufficiently low to not inhibit the normal functioning of the stomach. It would appear, therefore, that the action of the fat is not antagonistic to the commonly accepted methods of making the milk more digestible, such as diluting with lime water or barley water, or boiling. Though the abomasum of the calf being fed milk is rarely if ever empty, the normal amount of fat present in the milk will tend to produce a softer more friable curd which usually leaves the stomach more quickly than curd formed from skim milk.

#### SUMMARY

Although whole milk has always been considered preferable for calf feeding to separator skimmed milk from a growth promoting standpoint, the cost of feeding whole milk makes it desirable to change to skim milk feeding at the earliest possible age. In this paper the physiological effect of the fat on the rate of evacuation of the stomach is discussed. Curd from milk containing up to six per cent fat tends to leave the stomach more rapidly than skimmed milk due to the difference in texture of the curd formed. Milk containing this amount of fat does not apparently inhibit gastric secretion or gastric motility. Fat, in addition to being a valuable source of food, would appear to aid digestion when incorporated in the milk in limited amounts.

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# THE MECHANICAL CONTROL OF THE FAT CONTENT OF SWISS CHEESE

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The eye formation in Swiss cheese is one of the most important factors in determining its market grade. Inspectors commonly establish this grade by simply drawing and examining two or three plugs from a wheel of Swiss without either smelling or tasting the samples. Dealers justify this procedure by stating that the trade is chiefly interested in the eye formation and slicing properties and that other characteristics are of so little significance that they may be practically neglected in all except unusual circumstances. Connoisseurs of cheese, who are aware of this condition, believe that subordination of flavor and body to eye formation and slicing properties will eventually injure the sale of Swiss cheese.

Within a radius of 50 miles from Monroe, Wisconsin, are located nearly 200 Swiss cheese factories. During the season of greatest production in 1933, a study was made in this area to determine, under factory conditions, the relation of the mechanical control of the fat content of the cheese to the composition and commercial quality of the finished product.

The ideal formation of eyes in Swiss cheese depends upon the inter-related effects of biological and mechanical control of the curdmaking and ripening processes. Biological control is attained by using cultures of desirable organisms (1, 2, 3, 4) and directing their activities by regulating those factors which influence their life processes. Mechanical control is established by clarification and removal of fat from the milk (6) and by those manipulations in the curdmaking and ripening operations which assist in determining the composition of the finished product. The relation of these two general methods of controlling formation of eyes in Swiss cheese is such that, in factory practice, it may be impossible to state whether these manipulations are essential because they influence most the biological changes, the physical properties or the chemical composition of the cheese.

## METHODS

*Selection of factories.*—Twenty factories were selected for intensive study. They ranged in size from one to ten kettles and were located in the area approximately according to the intensity of production. Only those factories were selected in which the cheesemakers were recognized leaders in their art.

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*Samples.*—Each of these factories was visited at intervals of approximately one month. The visits began on June 20th and ended October 31st, 1933. Samples of milk, whey, curd, and cheese were taken at certain points in the making process that are designated in table 2.

*Methods of analysis.*—The Babcock test was used for determining the fat content of the various samples. Casein was measured by the Walker Formol Titration. Dry matter was found by drying the samples of curd and cheese at 100° C. until a constant weight was attained.

*Methods of grading.*—The grades of cheese were determined at approximately 9 weeks of age by representatives of the dealers who purchased them. Makers were always present when the buyer examined the cheese and could protest any unfair grade. Such disagreements arose very infrequently. There was no supervision of grading by any State official during the period of the study. The characteristics of the four market grades, which were recognized by the dealers, are classified in table 1. Grade I designates the best quality while a grade of IV indicates the most undesirable cheese and is commonly called a "grinder."

TABLE 1  
*Description of the market grades of cheese made between  
June 20, 1933, and October 31, 1933*

GRADE	I	II	III	IV
Eyes	Well Developed 1 to 5 per trier	Well Developed About 5 per trier	Overset	Blind
Texture	No Glass nor Pinholes	No Glass nor Pinholes	Glass No Pinholes	Pinholes
Body	Firm and Meaty	Fairly Firm and Meaty	Fairly Firm and Meaty	Fails to Meet Requirements for other Grades
Flavor	Clean, Desirable, Characteristic of Age	Clean, May Lack Flavor	May Lack Flavor, Not Objectionable	Fails to Meet Requirements for other Grades
Finish	Well-shaped, Clean, Sound Rind	Well-Shaped, Clean, Sound Rind	Clean, Well-shaped, Sound Rind, Slightly Damaged	Clean, Sound Rind, Slightly Damaged
Salt	Uniform	Uniform	Uniform	Uniform

#### RESULTS

A total of 189 wheels of Swiss were made under the observation of the laboratory representatives. Occasionally a cheese was sold before it could

be analyzed; this resulted in a final total of 176 wheels graded and analyzed. The summary of the analyses of the various samples is presented in table 2. Each sample represents the contents of a single kettle of milk, weighing approximately 2700 pounds, or its equivalent in whey, curd, or cheese.

TABLE 2  
*Statistical summary of analyses from twenty Swiss cheese factories in southern Wisconsin*

MATERIAL	NUMBER OF SAMPLES*	AVERAGE	PLACE AND TIME OF SAMPLING
WHOLE MILK			
Per cent fat	189	3.19 $\pm$ 0.010	Sampled as the milk
Per cent casein	189	2.3 $\pm$ 0.006	left the receiving
Ratio,—Casein — fat	189	0.72 $\pm$ 0.002	vat to be clarified
STANDARDIZED MILK			
Per cent fat	189	2.8 $\pm$ 0.009	Sampled in the cheese
Per cent casein	187	2.3 $\pm$ 0.006	kettle just before
Ratio,—Casein — fat	187	0.815 $\pm$ 0.003	adding rennet
WHEY			
Per cent fat	188	0.90 $\pm$ 0.008	Sampled in cheese
			kettle at dipping
CURD			
Per cent fat	189	25.0 $\pm$ 0.07	Sampled in cheese
Per cent dry matter	185	56.9 $\pm$ 0.08	
Per cent fat in dry matter	185	43.8 $\pm$ 0.10	
CHEESE			
Per cent fat	176	27.4 $\pm$ 0.06	Sampled at factory
Per cent dry matter	177	60.4 $\pm$ 0.05	
Per cent fat in dry matter	176	45.1 $\pm$ 0.09	

\* Each sample represents 2500 to 3000 pounds of mixed milk or its equivalent in whey, curd or cheese.

#### *Composition of the whole milk delivered to the Swiss cheese factories*

**Fat.**—Farmers in the Swiss producing area of Southern Wisconsin have never universally accepted the fat content of milk as a basis of payment. Returns are calculated commonly on the weight of milk delivered to the factory by the patrons. Breeding for production, rather than fat content, is undoubtedly responsible for the fact that the average fat content of the mixed milk delivered to many Swiss factories is actually less than 3 per cent during part of the year. Table 2 indicates an average fat content in the milk of 3.19 per cent. Figure 1 shows the distribution of the samples according to fat content.

**Casein.**—The average casein content of 2.3 per cent is slightly higher than might be expected with milk of this average amount of fat. The limit

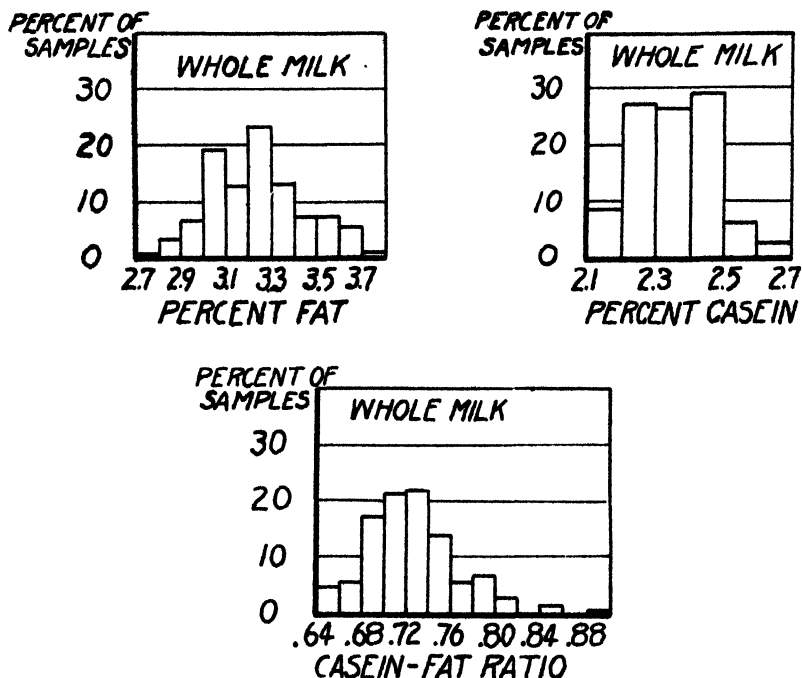


FIG. 1. Distribution of samples of whole milk classified according to the percentage of fat, percentage of casein and the ratio of casein to fat  $\left(\frac{\text{casein}}{\text{fat}}\right)$ .

of accuracy of the Walker test for casein, however, does not make this difference of any significance.

*Ratio of casein to fat.*—The ratio of casein to fat is calculated by dividing the percentage of casein by the percentage of fat in milk. Table 2 shows an average ratio of 0.72 for the milk delivered by the farmers. The highest value of this ratio observed was 0.89 per cent. It is astonishing but serves to emphasize the nature of the milk which is being used. Figure 1 illustrates the variability of the casein-fat ratio.

#### *Composition of the standardized milk used in making Swiss cheese*

It is known (5, 6) that the ratio of casein to fat in milk used for Swiss cheese has an influence on the formation of eyes. A high ratio produces better eye-forming properties in the curd. The adjustment of this ratio is called "standardization" and it is always accomplished by the removal of cream from the milk. The legal requirement of 45 per cent of fat in the dry matter of the cheese places a limit upon the extent of this adjustment.

The common factory practices for processing and standardizing the

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milk in preparation for cheesemaking should be mentioned. The whole milk, as soon as it is delivered by the farmers, runs or is pumped to the separator or clarifier. Clarifiers are not used ordinarily, although one was found in a factory included in this study. The usual separator in the Swiss factory does triple duty: it clarifies the milk, removes a portion of the fat at the same time, and later in the day is used to separate the fat from the whey. The separator serves as a clarifier by simply turning both cream and skimmilk spouts into a conductor trough which carries the mixture of milk and cream to the cheese kettle. The cream is diverted from the kettle long enough to establish the desired ratio of casein to fat in the kettle milk. The regulation of this removal of cream cannot be accurate, because it is commonly based neither on accurate tests of cream or milk for fat and casein, nor upon exact knowledge of the weight of the milk in the kettle, although sometimes the depth of skimmilk in the kettle may be measured before the whole milk is allowed to run into it.

Figure 2 presents a graphical summary of the analyses of the samples of standardized milk for fat and casein, and shows the calculated casein-fat ratio.

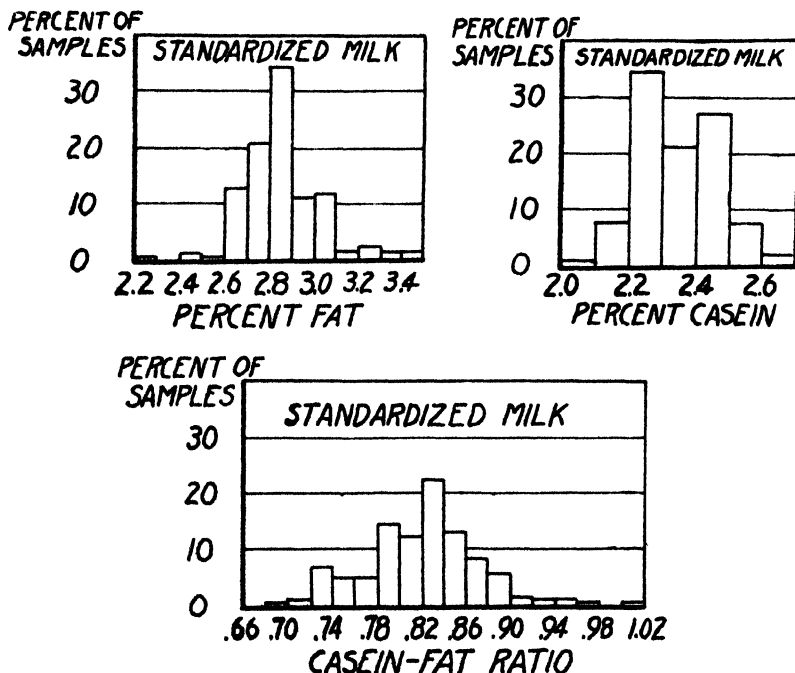


FIG. 2. Distribution of samples of standardized milk according to the percentage of fat, percentage of casein and the ratio of casein to fat  $\left(\frac{\text{casein}}{\text{fat}}\right)$ .

*Fat.*—There is a common belief among makers that a fat content of 2.8 per cent in the kettle milk makes good cheese. This may explain why the average of the samples approximates this value in table 2. Actually, however, the relative variability of the per cent fat in the standardized milk is practically the same as that of the fat in the whole milk.

*Casein.*—There is no measurable difference in the average casein content of the standardized milk and the whole milk, as is shown in table 2.

*Ratio of casein to fat.*—There is not a great deal of information available to indicate the most desirable ratio of casein to fat to establish in order to make cheese of high quality and legal composition. There are so many variable factors involved in the making process that perhaps specific recommendations are not justified. Matheson (5) recommends a ratio of casein to fat of 0.72 when the fast loss in the whey equals 0.9 to 1.0 per cent, and a ratio of 0.80 when the fat loss is 0.6 to 0.7 per cent. Peter (6, page 120) indicates that in general the cheese will contain 45 per cent of fat in the dry matter if the proportions of casein to fat are kept within the range of 0.73 to 0.75 depending upon the season of the year. He assumes that a normal loss of fat in the whey is 0.5 per cent.

In view of these recommendations it is interesting to observe in table 2 that the average ratio of casein to fat in the standardized milk is 0.815.

The data of this study indicate that the ratio of casein to fat in the standardized milk is related to the fat in the dry matter of the cheese by the correlation coefficient of  $-.38 \pm .04$ . Similar calculations show a correlation coefficient of  $.20 \pm .05$  between the fat content of the standardized milk and the fat in the dry matter of the finished product. Evidently the ratio of casein to fat in the kettle milk is more important in determining the fat in the cheese than the actual fat content of this milk. The fat in the milk would be more definitely related to the fat in the cheese were it not for the fat losses which take place in the whey.

#### *Loss of fat in the whey at the time of dipping*

The average loss of fat in the whey at the time the curd was removed from the kettle is 0.90 per cent. Despite the wide range of the observations, indicated by the maximum and minimum values in figure 3, there are only about 1/5 of the observations which differ from the average by more than 0.2 per cent.

Although the fat losses in the whey must be a major factor in determining the fat content of the finished cheese, still, calculations indicate that the loss of fat in the whey is related to the fat content of the cheese and to the fat in the dry matter of the cheese by correlation coefficients of approximately  $-.25 \pm .05$ . This does not mean that excessive losses of fat may not cause the production of cheese with low fat content. As a matter of fact,

# CONTROL OF THE FAT CONTENT OF SWISS CHEESE

only 8 of the 22 wheels of cheese made with fat losses in the whey exceeding 1.0 per cent, contained the minimum fat required by law.

The apparently insignificant effect of the fat losses in the whey upon the composition of the cheese may be explained by the fact that the fat losses in the whey increase as the fat content of the standardized milk increases. This relationship, which is expressed by the correlation coefficient of  $.58 \pm .03$ , is illustrated in figure 3. But, as the fat content of the standardized milk increases, the fat content of the finished cheese shows only a very slight tendency to increase. The correlation between these two variables is

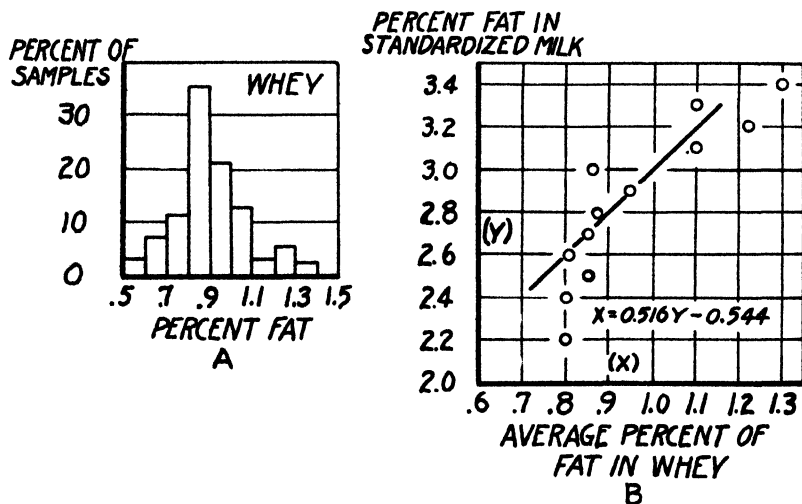


FIG. 3. A. Distribution of samples of whey classified according to the percentage of fat. B. Classification of the samples to show the average percentage of fat in the whey associated with unit changes in the fat content of the standardized milk.

only  $.21 \pm .05$ . Evidently, the escape of larger and larger amounts of fat in the whey, as the fat in the kettle milk increases, reduces the amount of fat available for the cheese to such an extent that the richer standardized milk actually fails to produce any marked increase in the fat content of the cheese.

The tendency toward higher fat losses in the whey with increasing amounts of fat in the standardized milk is undoubtedly exaggerated by the so-called "clarification" of the milk with separators. The treatment is more severe than clarification in its effect on the physical condition of the fat in the milk. When milk is processed in a clarifier, the fat which gathers at the center of the bowl, is redispersed in the skimmilk as it leaves the centrifuge. Milk, which has been properly clarified, can be made into cheese

without increasing the whey fat losses that would occur if the milk had not been clarified. (7). It is commonly known, however, that cheese made from skimmilk or partly skimmed milk to which cream has been added, shows a greater whey fat loss than would occur with whole milk of the same fat content. This loss increases as the proportion of cream in the mixture increases. It is not surprising, therefore, to discover fat losses in whey from Swiss cheese approximating 0.9 per cent, under the conditions of processing which have been described. The rapidity with which the observed losses of fat in the whey increase with increasing amounts of fat in the kettle milk is undoubtedly caused by partial separation followed by inadequate mechanical redispersion of the fat in the milk.

*Fat and dry matter in the curd at the time of dipping*

The samples of curd were taken from the kettle and were analyzed several hours later in the central laboratory. During this interval some whey drained from the curd. Since whey contains in solution approximately 6 per cent of dry matter not fat in the form of milk sugar, milk salts, and whey-soluble proteins, the proportion of fat in the dry matter, as well as the actual percentage of fat and dry matter, were changed by this uncontrolled loss of whey from the curd. This condition, as well as those normal differences in making procedures which exist in different factories, make it impractical from our data to attempt to predict the composition of the finished cheese from the analyses of the curd at dipping.

The average values of fat, dry matter, and percentage of fat in the dry matter of the curd, are summarized in table 2 merely to complete this record.

*Fat and dry matter in the cheese at the time of grading*

*Fat.*—The fat in the cheese averages 27.4 per cent. The variations in these observations are shown in figure 4. There is a general tendency for the fat in the cheese to increase as the ratio of casein to fat in the standardized milk decreases, as indicated by the correlation coefficient of  $-.35 \pm 0.04$ .

*Dry matter.*—The dry matter in the cheese averages 60.4 per cent. Practically 85 per cent of all the samples of cheese contain within 1 per cent of this amount of dry matter, as illustrated in figure 4.

The dry matter in the cheese depends upon those factors in the making process which affect the moisture content and the retention of fat in the curd. During the curing period, the amount of salting, the humidity and temperature of the rooms, and the length of time of holding influence the dry matter in the ripened cheese. Correlations calculated between dry matter in the cheese and such factors as milk composition and loss of fat in the whey were all insignificant.

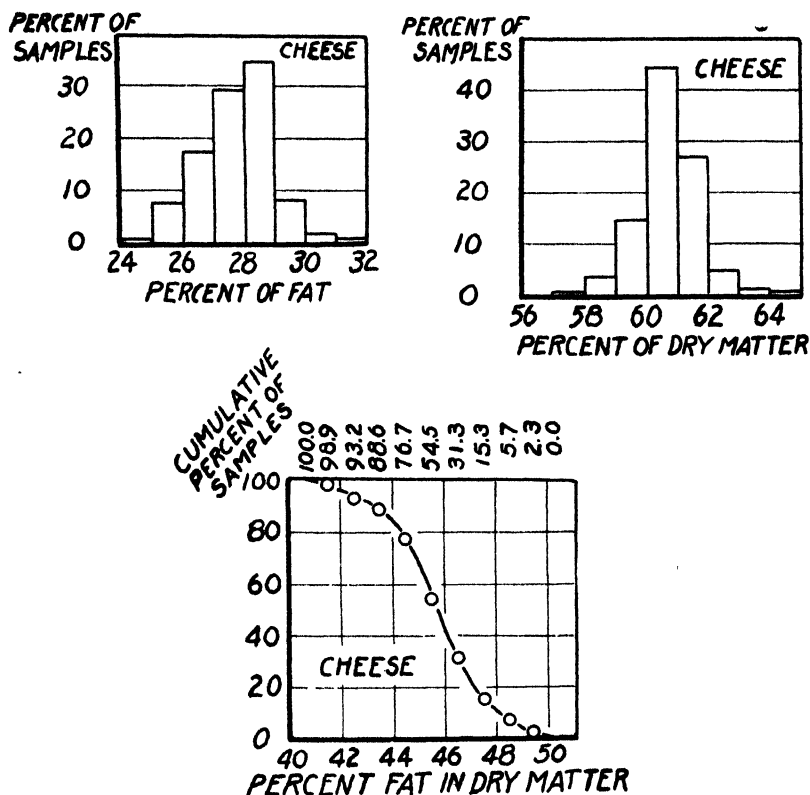


FIG. 4. Distribution of samples of cheese classified according to the per cent fat, per cent dry matter and per cent fat in the dry matter.

*Fat in the dry matter of the cheese.*--The percentage of fat in the dry matter of the cheese is calculated by dividing the percentage of fat by the percentage of dry matter and multiplying the result by 100. This factor is especially significant because it is used to define the legal composition of cheese. Figure 4 illustrates the variations in this calculated value for 176 wheels of cheese. This cumulative frequency distribution of the samples according to the percentage of fat in the dry matter of the cheese, indicates the proportion of the total number of samples which contain amounts of fat in the dry matter equal to or greater than any selected value shown on the horizontal axis. If, for example, 45 per cent is the chosen value, it is apparent that approximately 66 per cent of the samples contain at least this amount of fat.

The average percentage of fat in the dry matter, as shown in table 2, is 45.1. The standardization of the milk appears to be closely related to this

variable. As the ratio of casein to fat in the standardized milk decreases there is a fairly definite trend toward a higher percentage of fat in the dry matter of the cheese. This trend is expressed by the correlation coefficient  $-.38 \pm .04$  and is illustrated in figure 5, in which the ratios of casein to fat in the kettle milk have been averaged for each unit change in per cent of fat in the dry matter of the cheese.

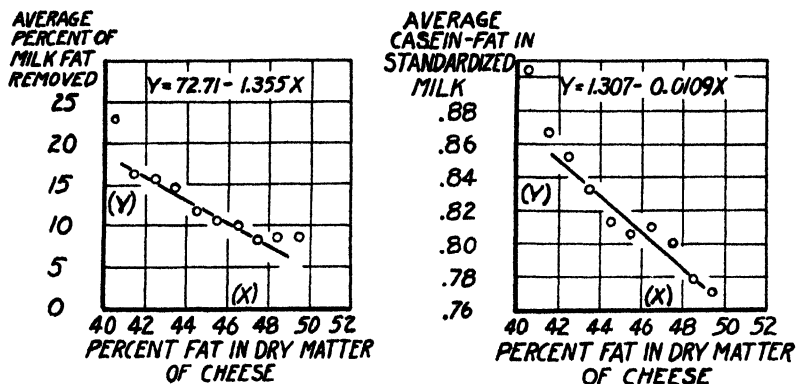


FIG. 5. Classification of the samples to show the average percentage of milk fat removed by standardizing and the average ratio of casein to fat  $\left(\frac{\text{casein}}{\text{fat}}\right)$  in the standardized milk that are associated with unit changes in the percentage of fat in the dry matter of the cheese.

Another indication of the amount of fat removed by standardizing the milk has been calculated for each kettle by taking the difference between the fat tests of the whole milk and the standardized milk and dividing this difference by the per cent of fat in the whole milk. The result, when multiplied by 100, might be regarded as "The percentage of milk fat removed by standardizing." Since the exact weights of the milk in the kettle and the cream removed from the kettle were not known, this calculation is obviously inaccurate. It may serve, however, to indicate the relative amount of skimming which took place in the factories and its effect on the composition of the finished cheese. This relationship, which is described by the coefficient of correlation  $-.46 \pm .04$ , is shown in figure 5 where the average "percentage of milk fat removed by standardizing" has been plotted for each unit change in the per cent of fat in the dry matter of the cheese.

Figure 5 indicates the average values of the ratio of casein to fat in standardized milk and "percent of fat removed," which are associated with certain amounts of fat in the dry matter of the cheese. The application of these values to determine adjustments of milk composition at the beginning of the making process must be attempted with caution. These values are

averages for many plants and the observations upon which they are based were made over several months. They only indicate the trends of the effects and dangerous limits of adjustments of milk composition. It seems reasonable to believe that the variability of the data from all the plants is greater than that which should obtain for similar measurement in any single plant where manufacturing conditions are more uniform from day to day. Careful measurements repeated each day in any given factory, therefore, should make it possible to establish more definite relationships than those indicated in figure 5.

### *The quality of the cheese*

The average of the grades of the lots of cheese produced during the study is 2.16.

Coefficients of correlation calculated between the grade and such variables as: fat in the milk delivered by the farmers, casein-fat ratio in standardized milk, and fat loss in the whey, any of which might be expected to influence the grade of the cheese, were all reasonable in trend but insignificant in value. As each factor tended to produce or to indicate a decrease in the percentage of fat in the dry matter of the cheese there was a slight tendency toward improvement in the quality of the cheese. The fat in the dry matter of the cheese is the only observed variable which seems to influence the grade. This relation is summarized in table 3 in which the average percentage of fat in the dry matter has been calculated for the cheese falling in each grade. As the grade moves from I to IV there is

TABLE 3  
*Relation between grade and percentage of fat in the dry matter of cheese*

GRADE OF CHEESE	NUMBER OF CHEESE	AVERAGE PER CENT FAT IN DRY MATTER OF CHEESE
I	68	44.5
II	49	44.8
III	23	45.1
IV	36	46.7
Total	176	

a gradual increase in the average fat content of the cheese. This relationship is described by the coefficient of correlation  $.42 \pm .04$ .

### DISCUSSION

The graders of Swiss cheese act essentially as the interpreters of trade demands because they are employed by those who market the cheese. The premium paid for the highest quality of Swiss cheese is attractive enough to determine the trends of factory practices. Since the best grades seem to be associated with less fat in the dry matter of the cheese it is natural to expect the makers to reduce the fat in the finished product to the minimum.

Although trade demands are rightfully interpreted by those who merchandise Swiss cheese, it is reasonable to question the influence on the dealers' opinions of the demands of the large buyers who desire cheese with good slicing properties and large eye formation. A survey of the industry and public opinion would be necessary to determine whether these characteristics are more important to the ultimate consumer than the mellowness of body and superior flavor which can be best attained by retaining a larger proportion of the milk fat in the cheese.

There are other factors, outside the scope of this investigation, that influence, directly or indirectly, the composition and quality of the cheese, such as: milk quality, the use of milk from diseased udders, and the culture methods of controlling fermentations in the cheese. It is not known how much improvement in market grades is possible by regulating these factors and maintaining at the same time a mechanical control of cheese composition which would incorporate maximum amounts of fat. Information obtained by the cooperative efforts of the United States Bureau of Dairy Industry and the University of Wisconsin during the past several years indicate that milk of better quality and application of improved methods of using the available cultures definitely raise the market quality of the cheese.

It is evident, therefore, that any program of composition regulation, especially when such regulation must raise the fat content of the finished cheese, should also be accompanied by increased efforts to control the quality of the milk and the types of organisms in the cheese.

#### CONCLUSIONS

Mechanical control of the fat content of Swiss cheese depends upon accurate standardization. Simultaneous clarification and separation of milk, excessive losses of fat in the whey, and milk with abnormally low fat content makes such standardization a difficult procedure in some plants.

The removal on the average of more than about 10 per cent of the total fat from the lots of milk observed in this study or the establishment of casein to fat ratios greater than approximately 0.81, tend to produce cheese with less than the legal amount of fat. Since unusual biological conditions in the milk or special methods of curdmaking may cause variations in cheese composition, it is essential that every plant operator analyze the milk, whey, and cheese from every kettle before accepting these average values as actual standards.

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## RUMINANT DIGESTION WITHOUT ROUGHAGE

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In a study of the value of roughage in the dairy ration, several heifers have been maintained at this station on a roughage-free diet from birth to over 18 months of age. A similar group was reared on the same mixture of concentrates plus approximately 14 per cent of sulphite wood paper pulp. During this work, digestion trials were conducted with heifers from each group. The results are here reported.

### PROCEDURE

Three digestion trials were conducted, four heifers being used in each. The animals received the same feed mixtures that they had been receiving since two months of age except for the mixture used in trial 3. During this trial and for ten days before the collection period, they received mixture 3, which differed from mixture 1 only in being more finely ground. This trial was intended to determine the effect of fine grinding on digestibility: mixture 2 was more finely ground than mixture 1, because of the method of preparation necessary to incorporate the paper pulp. The heifers received daily a medicinal cod liver oil, the total amount of which is shown in table 2. The compositions of the feed mixtures are shown in table 1.

TABLE 1  
*Percentage composition of feed mixtures*

FEED MIXTURE NO.	INGREDIENTS						
	Ground barley	Wheat bran	Soybean meal	Sodium chloride	Calcium carbonate	Calcium citrate	Paper pulp
1	61.5	26.3	8.8	1.9	1.5		
2	52.0	22.2	7.4	1.6		3.0	13.8
*3	61.5	26.3	8.8	1.9	1.5		

\* Identical in composition with feed mixture 1 but more finely ground.

The animals used were grade Holstein heifers, 18 months of age, which had received since birth a ration devoid of roughage. They were normal in size for the breed and showed no abnormalities other than frequent bloating and lack of regular ruminations, both of which have been found to be common with ruminants receiving this type of ration. According to analysis, the gas causing bloat consisted largely of carbon dioxide with no methane.

During the digestion trials the daily amounts of feed for each animal

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were reduced, since animals usually consume less when standing in stanchions than when exercising in the corral.

The heifers were placed in the digestion stalls 6 days before the collection of feces. The collection period lasted for 10 days. Heavy gymnasium mats replaced the common forms of bedding. The animals were fed and watered thrice daily. Each day, the total feces collected during the previous 24 hours were spread out in drying trays and placed in a drying oven maintained at a temperature of 90° to 95° C.<sup>1</sup> A period of 48 hours was required to dry each day's collection. When dry, the feces from each animal were placed in separate tightly covered cans. At the end of the collection period the dried feces were weighed, and a sample was taken for moisture. The remainder was ground in a hammer mill, thoroughly mixed, and sampled for chemical analyses.

#### PRESENTATION OF DATA

The amounts of feed consumed and feces collected are shown in table 2. Chemical analyses of feed mixtures and feces are presented in tables 3 and 4; coefficients obtained, in table 5.

TABLE 2  
*Total food consumed and feces collected (grams)*

ANIMAL NO	TRIAL 1			TRIAL 2			TRIAL 3		
	Mixture 1	Cod liver oil	Feces (dry weight)	Mixture 2	Cod liver oil	Feces (dry weight)	Mixture 3	Cod liver oil	Feces (dry weight)
34	40,800	558	8,220				36,300	558	7,180
35	58,860	1116	10,870				43,800	1116	9,560
37	45,400	558	8,740				40,800	558	9,010
39	54,400	558	11,050				45,400	558	10,710
41				56,410	630	15,200			
43				40,800	558	8,160			
46				36,300	558	10,410			
47				36,300	558	7,580			

During the three digestion trials all but two of the animals cleaned up their feed well. Number 35 left 137 grams of the last feeding of trial 1; and during trial 3, when receiving the finely ground feed, she had an irregular appetite and refused a total of 1,690 grams. Number 41 left 2,590 grams of feed during trial 2. The daily ration of mixture 3 (table 2) was less than had been allowed the same animals in trial 1, in anticipation of a decreased consumption. While receiving this finely ground feed before the

<sup>1</sup> While this paper was in preparation, D. P. Cuthbertson and A. K. Turnbull, (Biochem. J. 28: 837, 1934) found a loss of 4 to 5 per cent of nitrogen in drying human feces on the steam bath. In our laboratory, R. W. Caldwell has confirmed these findings: The nitrogen loss was 3 to 4½ per cent in drying freshly voided bovine feces at 100° C., for 48 hours in an electric oven.

TABLE 3  
Chemical analyses of feed mixtures, per cent

ANIMAL NO.	TRIAL 1		TRIAL 2*				TRIAL 3	
	34-35	37-39	41	43	46	47	34-35	37-39
Moisture	10.3	10.7	9.0	9.0	9.5	8.4	11.8	11.1
Protein	14.7	14.0	12.0	12.2	12.3	12.2	13.9	13.9
Nitrogen-free extract	60.0	61.0	54.9	56.1	55.4	56.1	60.3	60.4
Ether extract	1.3	1.4	0.7	0.7	0.8	0.8	1.3	1.3
Crude fiber	6.9	6.5	17.6	16.3	16.1	16.7	6.5	7.2
Ash	6.8	6.4	5.8	5.7	5.9	5.8	6.2	6.1

\* Because of the physical nature of mixture 2, separate samples of this feed were analyzed for each animal.

TABLE 4  
Chemical analyses of feces collected, per cent dry basis

ANIMAL NO.	TRIAL 1				TRIAL 2				TRIAL 3			
	34	35	37	39	41	43	46	47	34	35	37	39
Protein	13.2	12.7	14.8	12.4	10.2	13.3	11.5	11.2	12.7	11.7	12.4	12.3
Nitrogen-free extract	47.0	45.2	45.9	46.4	38.5	44.1	42.9	44.0	47.8	48.5	48.6	47.4
Ether extract	2.5	3.3	2.7	2.6	2.4	2.3	3.0	2.5	3.0	2.2	2.9	2.6
Crude fiber	20.6	20.8	21.0	22.4	38.9	27.9	31.4	29.8	22.0	23.5	23.0	23.3
Ash	16.7	18.0	15.6	16.2	10.0	12.4	11.2	12.5	14.5	14.1	13.1	14.4

TABLE 5  
Coefficients of digestibility

DRY MATTER				PROTEIN			NITROGEN-FREE EXTRACT			ETHER EXTRACT			CRUDE FIBER		
TRIAL NO.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Animal No.															
34	78		78	82		82	84		84	81		79	40		33
35	80		76	84		82	86		82	81		88	44		21
37	79		76	80		80	86		82	80		76	38		30
39	78		74	82		79	85		82	78		76	30		24
41		71			77			81			64			40	
43		78			78			84			78			66	
46		69			73			78			63			44	
47		78			81			84			78			63	
Average	79	74	76	82	77	81	85	82	83	80	71	80	38	53	27
Calculated	*82			86			88			81			56		

\* See text page 6.

digestion trial, the heifers apparently found it less palatable than the same mixture coarsely ground. Their feces were drier than with the coarsely ground mixture 1. A dose of one-half pound of Epsom salts had to be administered to heifer 35 on the fourth day of the collection period; thereafter her appetite improved somewhat, so that she cleaned her feed up fairly well at each feeding.

During trial 1 all the animals ruminated occasionally. In trial 2, No. 41 and No. 43 ruminated, but not regularly. No. 46 and No. 47, receiving less feed, were not observed to ruminate at any time. During trial 3, with the finely ground feed, no rumination was noticed.

The paper pulp was originally added to the basal concentrate mixture to serve as an artificial roughage, somewhat less complex than natural roughage. Rumination occurred only occasionally, however, no more in one group than another, even though concentrate mixture 1 contained 6.8 per cent crude fiber, whereas the same mixture plus paper pulp, mixture 2, contained 16.7 per cent.

TABLE 6  
*Water consumption and number of defecations and urinations during each ten-day collection period*

ANIMAL NO.	DEFECATIONS	URINATIONS	WATER CONSUMED (POUNDS)
Digestion Trial 1			
34	63	104	350
35	44	58	483
37	68	73	330
39	63	70	332
Average	60	76	374
Digestion Trial 2			
41	76	36	350
43	76	55	251
46	49	27	201
47	50	46	208
Average	63	41	253
Digestion Trial 3			
34	59	92	330
35	49	61	200
37	66	65	351
39	78	69	308
Average	63	72	297

The amounts of water consumed, with the number of defecations and urinations, are reported in table 6. During a ten-day metabolism trial in connection with another experiment, nine cows receiving a normal ration of concentrates and hay averaged 11 defecations and 5 urinations daily. In

these cases there was a variation of 9 to 15 defecations and 3 to 7 urinations. The number of defecations was almost double that recorded for the heifers used in the digestion trials here reported; the number of urinations, about the same.

#### DISCUSSION

As roughage has been considered essential in the diet of ruminants, the accepted procedure for determining the coefficient of digestibility for a given concentrate has been first to determine the digestibility of a roughage, which is later fed in combination with the concentrate, second to determine the digestibility of the combination ration, and third to calculate the difference between the two. Whether or not the results so obtained would be similar to those for the concentrate if fed alone has been considerably debated, though the usefulness of the information is not questioned.

The literature describes several investigations that have increased our knowledge regarding the effect of one feed or nutrient upon another. Since Titus (1), in discussing "the mutual influence of the proportion of the several nutrients, in feeds, on their digestibility," has reviewed this literature very completely, only investigations particularly pertinent to this discussion will be mentioned here.

We have failed to find any data concerning the digestibility of concentrates when fed without roughage for an extended period. In the investigations conducted by others, the concentrates were fed alone only for a relatively short time, doubtless because roughage has been considered essential. As experiments at this station (2) (3) have shown, however, dairy animals can be reared to four years of age on a concentrate diet provided it is supplemented with calcium carbonate and vitamin A.

According to certain evidence, the digestibility of a diet may vary with the length of time it is fed. This might be especially true should the diet be unusual—for example, composed entirely of concentrates. In this connection the work of Lyman (4) is interesting. He has shown that the continued use of white flour, in the diet of rats, caused a slight decrease in protein utilization. The continued use of whole wheat meal in a similar food combination, however, decidedly improved the utilization of this nutrient.

The coefficients of digestibility determined for mixture 1 (concentrates only) have been compared in table 5 with calculated values for this mixture when fed with roughage, arrived at by using the coefficients of each ingredient in the mixture as given by Henry and Morrison (5). The coefficients of digestibility for the dry matter and the ether extract of cod liver oil were both assumed to be 90 per cent, that of the dry matter of soybean-oil meal 92 per cent, and that of salt 100 per cent. The digestibility of calcium carbonate was disregarded. Except in the case of crude fiber,

which was 32 per cent lower, the coefficients actually determined were not significantly below those calculated.

Admittedly, the coefficients of digestibility used in calculating values are based upon a relatively few trials; and nutrients existing in comparatively small amounts, such as the crude fiber in our mixture 1, may give erroneous results. The heifers used in trial 1, however, did not ruminate regularly, a fact that may explain the apparent low digestibility of the crude fiber. As Ewing and Wright (6) have shown "More than half of the comminution that takes place in average rations containing coarse feeds takes place as a result of mastication." In an earlier publication, Ewing, Wells, and Smith (7) showed that a steer would macerate his food more completely when fed silage alone than when fed silage and cottonseed meal, the extent of maceration decreasing as the proportion of meal increases; and that the loss in digestibility of the crude fiber, which resulted from a combination of silage and cottonseed meal, was caused by a less complete maceration.

Mixtures 1 and 3 were identical except that the latter was more finely ground. The digestibility of mixture 3 was determined in order that the results obtained for mixtures 1 and 2 would be on a more comparable basis, since mixture 2 was necessarily more finely ground than mixture 1. In this trial (table 5), mixtures 1 and 3 showed no significant difference in digestibility except in crude fiber. Not only was the average coefficient of digestibility for this ingredient in mixture 3 lower, but none of the animals showed so high a value for this nutrient in trial 3 as in trial 1. The results, therefore, appear significant. This decrease might be explained by the work of Ewing and Smith, (8) who, having studied the rate of passage of food residues through the steer, concluded that finer-ground feeds pass through the animal more rapidly and that the digestibility of crude fiber seems to decrease with a more rapid passage of feed residues.

According to trial 3, any differences in digestibility between mixtures 1 and 2, except for crude fiber, cannot be explained on the basis of fine grinding.

When the coefficients of digestibility for mixture 1, consisting entirely of concentrates, are compared with mixture 2, containing 14 per cent paper pulp (table 5), the digestibility of all nutrients except fiber is seen to decrease slightly. These decreases, however, are probably not significant.

The digestibility of the crude fiber in mixture 2 was considerably higher than that in mixture 1 and approximately the same as the calculated value for mixture 1 when fed with roughage, doubtless because paper pulp itself has a high coefficient of digestibility. In two trials with oxen, Kellner (9) found the digestibility of crude fiber in paper pulp obtained by chemical treatment of rye straw to be 94.7 and 97.0 per cent. To explain his results he calls attention first to the fineness of the fibers in the paper pulp and

second to the fact that the chemical treatment had removed the incrusting substances which normally interfere with its digestion.

The digestibility of the crude fiber in the sulphite pulp used in our trials, calculated indirectly from our data, was lower than Kellner reported, but higher than the digestibility of the crude fiber in hays.

As the paper pulp in mixture 2 represented two-thirds of the fiber content of this mixture, the increased digestibility of the fiber in mixture 2 can be explained by the high digestibility of the paper pulp fiber.

#### SUMMARY

Digestion trials were conducted with Holstein heifers that had been fed for 18 months on a ration devoid of roughage, and with a second group receiving the same diet plus paper pulp. The effect of fine grinding on digestibility was also investigated.

Except for crude fiber, which was 32 per cent lower, the digestibility of a ration consisting entirely of concentrates was not significantly below the calculated value for the same mixture fed with roughage.

The addition of paper pulp to the concentrate diet, so that the fiber content of the ration was equivalent to that of one containing equal parts of concentrates and alfalfa hay, increased the apparent digestibility of the crude fiber. The digestibility of the other nutrients did not appear to be significantly altered.

It is not to be concluded that the addition of paper pulp enhanced the digestibility of the fiber contained in the concentrates. The higher value found for fiber in the paper-pulp ration may be explained by the fact that the fiber of paper pulp, as shown by other investigators, is more highly digestible than that of either roughage or concentrates.

Fine grinding appeared only to lower significantly the digestibility of the crude fiber of the concentration ration.

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# THE USE OF SODIUM THIOSULPHATE DILUTION BLANKS IN DETERMINING THE GERMICIDAL EFFICIENCY OF CHLORINE STERILIZERS\*

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Although chlorine compounds are widely used as sterilizing agents for dairy and milk plant equipment, their use is not sanctioned by all regulatory agencies nor are all investigators in agreement as to their effectiveness in destroying microorganisms.

Mudge and Smith (2) present a critical discussion of the effectiveness of chlorine compounds as dairy sterilizers and raise the question as to whether bacteria are killed or merely inhibited in growth by the action of these compounds. Furthermore, they question the value of plate counts as made by the usual laboratory methods for determining the germicidal efficiency of chlorine sterilizers. They point out that the results obtained by plate counts are a measure of the inhibiting action rather than a measure of the lethal effect of the chlorine compound. They express the opinion that, when a solution containing a chlorine compound is added to the peptone agar, the chlorine remains as a potent organic chloramine capable of inhibiting subsequent bacterial multiplication. To prevent the bacteriostatic action of residual chlorine they suggest the neutralization of this chlorine by the addition of sodium thiosulphate to the plate at the time of plating. They obtained, in general, a greater colony count with plates to which sodium thiosulphate had been added than with plates not receiving this treatment.

In addition to demonstrating the neutralizing effect of sodium thiosulphate, Mudge and Smith present evidence to show the effect of dilution of the residual chlorine carried over into the plate on the reduction of the bacteriostatic action. They report a colony count many times higher in the 1-10 than in the 1-1 dilution. They found this phenomenon to be the rule whether they used hypochlorite or monochloramine in high or low concentrations and with or without additions of sodium thiosulphate.

Devereux and Mallman (1) present data to show the value of sodium thiosulphate in determining the effectiveness of chlorine sterilizers against organisms in dairy equipment. Additions of sodium thiosulphate at the time of sampling neutralizes the chlorine thus making it possible to ascer-

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tain the true bacteriological status of the solution at the time of sampling even though immediate plating is not possible.

In studying the germicidal efficiency of a disinfectant it is necessary that the period of exposure of the disinfectant to the test organism be under definite control. Methods that do not permit of such a control because of the bacteriostatic action of the residual disinfectant are likely to give misleading results. In making plate counts of chlorine solutions the most desirable place to neutralize the residual chlorine is in the dilution blank. Accordingly the present investigation was planned to determine to what extent the presence of sodium thiosulphate in the dilution blank is of value in preventing the bacteriostatic action of the chlorine as shown by the subsequent colony count. Studies also were made of the effect of dilution in reducing the bacteriostatic action of the residual chlorine as shown by the numbers of colonies developing in plate cultures made from inoculated chlorine solutions.

#### METHODS

Dilution blanks of convenient volumes containing sodium thiosulphate were prepared as follows: To 50 cc. of M/5  $\text{Na}_2\text{HPO}_4$  was added 9.0 cc. of N HCl and the solution made up to 200 cc. with distilled water. To this buffered solution was added a 1.0 per cent solution of sodium thiosulphate at the rate of 10.0 cc. per 90.0 cc. after which the dilution blanks were prepared and sterilized. Preliminary experiments had shown this concentration of sodium thiosulphate in a 9.0 cc. dilution blank to be more than enough to neutralize the chlorine carried in a 1.0 cc. charge of the most concentrated chlorine solution used in this study and yet not interfere with subsequent colony growth. The resulting dilution blanks had a pH value of approximately 6.4. At this pH the reaction of the dilution blank is not deleterious to the test organisms and is favorable to the rapid reactivity of sodium thiosulphate and chlorine.

Plate cultures using plain agar were made immediately, 2.5, 5.0, 7.5, 10 and 15 minutes after inoculating the chlorine solution with the test organism. These cultures were plated simultaneously by two operators when dilution blanks with and without sodium thiosulphate were being compared. Plate cultures were incubated at 37° C. usually for a period of forty-eight hours.

Test organisms consisted of twenty-four hour old cultures of *Escherichia coli*, *Aerobacter aerogenes*, *Staphylococcus albus*, *Staphylococcus aureus* and *Streptococcus lactis*. Milk in which fermentation had taken place also was used.

The chlorine sterilizer used was a commercial preparation in which the active ingredient was calcium hypochlorite.

TABLE 1  
Comparative plate counts of inoculated chlorine solutions made with and without sodium thiosulphate dilution blanks

P.P.M. CL.	TEST ORGANISM	BACTERIA PER CUBIC CENTIMETER AFTER											
		Immediately		2.5 Minutes		5 Minutes		7.5 Minutes		10 Minutes		15 Minutes	
		With	With- out	With	With- out	With	With- out	With	With- out	With	With- out	With	With- out
21	<i>Esch. coli</i>	43,000	47,000	40,500	10,600	7,250	550	550	35	0	0	0	0
51	<i>Esch. coli</i>	101,000	70,000	12,600	10,400	700	140	20	0	0	0	0	0
98	<i>Esch. coli</i>	10,300	12,150	95	10	0	0	0	0	0	0	0	0
38	<i>Staph. aureus</i>	4,700	2,650	115	35	10	0	0	0	0	0	0	0
59	<i>Staph. aureus</i>	40,000	45,000	5,000	5,000	1,200	1,050	120	90	0	0	0	0
33	<i>Staph. albus</i>	3,500	2,050	750	950	450	125	30	20	25	15	15	30
31	<i>Staph. albus</i>	7,750	7,700	5,250	5,850	4,300	4,700	2,800	3,300	2,350	2,350	480	50
42	<i>S. lactis</i>	1,450	2,200	1,550	600	450	350	0	0	0	0	0	0
18	<i>S. lactis</i>	5,100	6,050	500	150	300	50	250	10	0	0	0	0
63	<i>Aer. aerogenes</i>	32,000	38,000	24,000	18,800	4,200	2,400	580	24	40	0	0	0
84	<i>Aer. aerogenes</i>	21,000	24,000	4,000	2,900	80	0	0	0	0	0	0	0
22	Milk	5,450	4,400	500	160	585	40	220	0	0	0	0	0
75	Milk	72,000	68,000	21,000	7,000	12,800	420	830	0	0	0	0	0
100	Milk	44,000	38,000	11,800	4,200	800	90	80	20	40	20	10	10
40	Milk	52,000	34,000	38,000	24,000	9,500	1,800	1,100	320	60	40	20	20
60	Milk	7,200	7,200	3,900	2,900	1,200	450	0	0	0	0	0	0

## PRESENTATION OF DATA

In table 1 are shown the results of sixteen comparative plate counts of inoculated chlorine solutions in which simultaneous platings were made using dilution blanks with and without sodium thiosulphate. Colony development, after brief periods of exposure of the organisms to the action of chlorine, up to 7.5 minutes and depending on the chlorine concentration, showed in most cases an increased count with the use of sodium thiosulphate in the dilution blank. However, when the organisms were exposed to the action of chlorine for periods of ten minutes or more, sodium thiosulphate had little or no effect on the subsequent colony development. The differences in numbers after brief periods of exposure to chlorine, while not great, show that bacterial cells which otherwise would be destroyed or inhibited in growth by the residual chlorine are able to continue normal development when freed of this chlorine. This is a factor that should be considered in evaluating the true germicidal efficiency of a chlorine sterilizer.

In the course of this study many plate counts were made in an effort to determine the influence of dilution on the bacteriostatic action of residual chlorine as shown by the numbers of colonies developing on plates of different dilutions. In no case was it found that more colonies developed on the plates of the higher dilutions than on plates of the lower dilutions. In this respect these results are not in agreement with those of Mudge and Smith (2).

## SUMMARY AND CONCLUSIONS

Simultaneous platings of inoculated chlorine solutions immediately after inoculating and after 2.5, 5.0, 7.5, 10 and 15 minutes, using ordinary dilution blanks and dilution blanks containing sodium thiosulphate were made and compared. In most cases an increased colony count resulted when sodium thiosulphate dilution blanks were used. Colony development following brief periods of exposure of the organisms to the action of chlorine showed, in most cases, an increased count when sodium thiosulphate dilution blanks were used. After longer periods of exposure, ten or more minutes, the presence of sodium thiosulphate had little or no effect on subsequent colony development.

The results of these experiments show that plate counts, in which ordinary dilution blanks have been used are likely to be misleading in evaluating the germicidal efficiency of chlorine sterilizers.

In an effort to study the effect of dilution in the bacteriostatic effect of the residual chlorine, plate counts of inoculated chlorine solutions, using ordinary dilution blanks, were made and the numbers of colonies developing on the plates of the 1-1, 1-10 and 1-100 dilutions compared. In no instance were more colonies found to develop on the plates of the higher dilutions than on plates of the lower dilutions.

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# A METHOD FOR THE DETERMINATION OF THE RELATIVE STIFFNESS OF CREAM DURING THE WHIPPING PROCESS\*

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The purpose of this paper is to describe a simple method for measuring the stiffness of cream during the whipping process and to show the importance of such measurements.

Since most consumers judge the quality of whipped cream by its consistency or stiffness, this property should be measured in studies of cream whipping. In the majority of such studies reported, manually operated whippers have been used and no provision has been made for measuring the consistency of the cream during whipping nor of the finished product. Babcock<sup>1</sup> describes a method for determining the relative stiffness of whipped cream. His method is not, however, applicable to uninterrupted measurements throughout the whipping process. So far as the writer knows, Templeton and Sommer<sup>2</sup> were first to report studies in which a mechanical whipper of constant speed was used and in which some provision was made for determining the stiffness of the cream during the whipping process. These investigators measured the consistency of the whipped cream by observing the torque (load) on the drive shaft of the turbine whipper.

## DESCRIPTION OF APPARATUS

The apparatus described herein consists of the mechanical whipper (restaurant size) and sensitive wattmeter, shown in figure 1. With this apparatus it is possible to obtain a continuous record of the stiffness of the cream throughout the whipping process by simply recording the input of the motor in watts at intervals of ten seconds, or less if desired. This method for measuring the stiffness of the cream in watts cannot, however, be used with all mechanical whippers, as the writer found in preliminary experiments with several small household whippers. The writer learned also that another investigator had also attempted in vain to use the wattmeter for measuring the relative stiffness of the cream as it was being whipped. These failures, no doubt, were due to certain characteristics of the mechanical whippers used. Furthermore, in order that small changes in load will be reflected in large changes in input, a motor should be used the maximum output of which is not much greater than the maximum

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<sup>1</sup> Babcock, C. J. 1922. U. S. Dept. of Agric., Bul. 1075.

<sup>2</sup> Templeton, H. L., and Sommer, H. H. 1933. Jour. of Dairy Sci., xvi, no. 4, 330

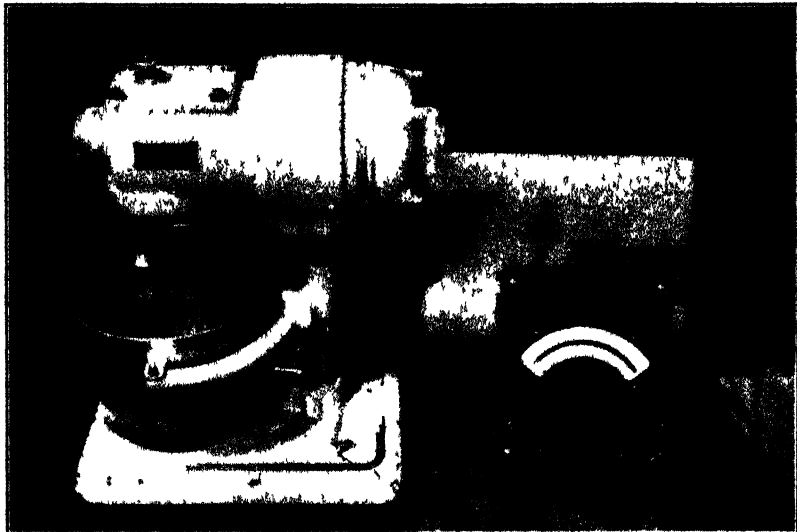


FIG. 1 Mechanical cream whipper and wattmeter (Gem kitchen mechanic whipper manufactured by Gem Appliances, Inc., 280 Madison Ave., N. Y., and a Westinghouse type PY5 portable single phase wattmeter).

required to whip the cream. If the normal output of the motor is relatively large, and the changes in load very small, these small changes in load will be insufficiently indicated by the variations in input as measured by the ordinary wattmeter.

The whipper-motor (figure 1), of which the input is charted, is a split-phase motor rated  $\frac{1}{2}$  HP and 1725 RPM. This motor produces in the whipper proper two distinct kinds of rotation. First, it spins the whipper on its vertical axis counter clockwise at a speed of 570 RPM. Second, it moves the spinning whipper (and its axis) clockwise about the inner periphery of the bowl so that the whipper completes 78 circuits about the bowl each minute. The speed of the whipper was checked under all loads which are normally encountered when whipping cream up to 40 per cent butterfat, and was found to be practically constant. Although the whipping bowl has a 5-quart capacity (liquid measure), only one quart of cream is usually placed in the bowl for whipping. The smallest scale division on the wattmeter is 5 watts, but 2.5 watts can be readily estimated.

That this wattmeter method is satisfactory to determine the relative stiffness of whipping cream at various times during whipping is proved in figure 2, in which appear typical curves obtained by plotting input against time when whipping cream of varying percentages of butterfat.

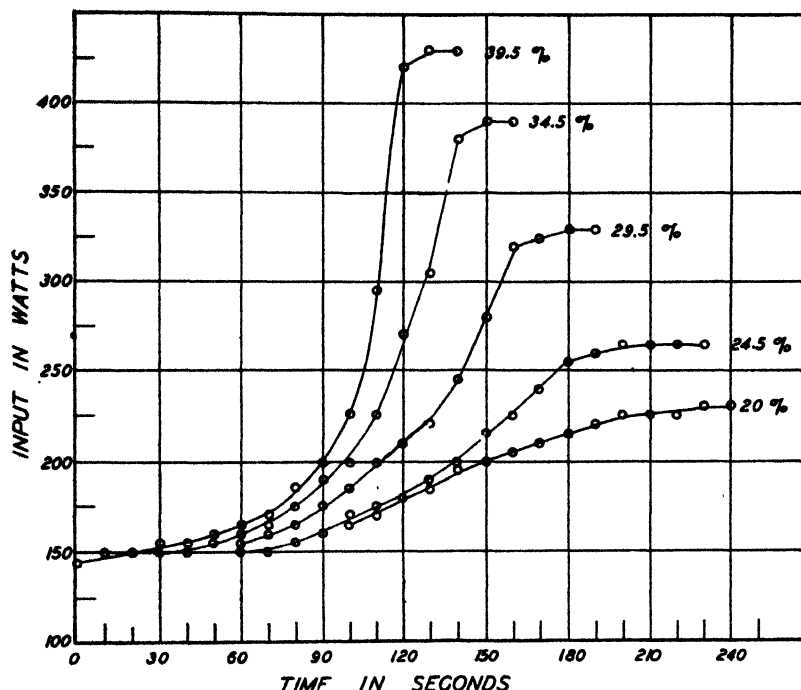


FIG. 2. Effect of fat content of the cream on the stiffness of the whipped cream.

#### APPLICATION OF METHOD

The wattmeter method for measuring the stiffness of whipping cream while the cream is being whipped should be useful in studies of cream whipping made either by the scientist or by the commercial dairyman. Measurements obtained by this method are certainly more accurate than visual observations and will serve several purposes. One use, for example, is partly illustrated in table 1, in which is indicated the effect of whipping a 33 per cent cream for varying periods of time on stiffness, overrun, amount of drainage, and percentage of butterfat in the drainage. It should be noted that at certain stages of the whipping procedure a variation of fifteen seconds in whipping time will materially affect the stiffness, the overrun, and the drainage of the whipped cream. A number of trials showed that the maximum overrun of the whipped cream occurs shortly before the maximum stiffness. It was found that the amount of drain decreased to a certain point with progressive whipping, reaching a minimum after the cream had been considerably over-whipped; but that upon still further whipping, it began to increase. The percentage of butterfat in the drain decreased with increased whipping until the cream was over-whipped, and

TABLE 1  
*Effect of whipping for varying periods of time on some of the properties of whipped cream*

WHIPPING TIME		STIFFNESS	OVERRUN	DRAIN AFTER 24 HOURS	FAT IN DRAIN	REMARKS ON BODY OF WHIPPED CREAM
<i>minutes</i>	<i>seconds</i>	<i>watts</i>	<i>per cent</i>	<i>cc</i>	<i>per cent</i>	
Start		125				
1	30	147.5	71.8	(1)		Too soft
1	45	155	75.6	(1)		" "
2	0	165	79.1	(1)		" "
2	15	182.5	85.6	57	6.84	Sl. too soft
2	30	225.0	94.8	37	2.19	Good
2	45	265.0	94.8	23	1.34	"
3	0	262.5	85.1	20	1.23	Sl. soggy
3	15	250.0	74.7	19	1.38	Soggy
3	30	250.0	68.5	16	1.52	"
4	0	240.0	57.3	18	1.25	Very soggy
5	0	230.0	35.5	27	1.17	Buttery

Cream used in this experiment contained 33 per cent of butterfat and was aged for 48 hours at 38° F.

(1) Too soft to remain on 12 mesh wire screening.

then remained fairly constant despite further whipping. It may be concluded, therefore, from the data in table 1 that it is very difficult to make accurate comparisons in cream whipping studies unless the consistency of the cream is accurately measured during the whipping process.

Accurate measurements of the consistency of cream during whipping are of practical value to the dairyman who sells whipping cream, because by the use of them he can set a standard for his cream and then check his cream each day in accordance therewith. If, then, the dairyman equips himself with a mechanical whipper and a wattmeter, he is able to produce a more uniform whipping cream or the finished product from day to day than he possibly could by unscientific means.

# NUTRITIONAL ANEMIA, CALCIUM PHOSPHORUS AND NITROGEN BALANCE AND BONE COMPOSITION OF RATS FED RAW VERSUS PASTEURIZED MILK\*

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## PART I. NUTRITIONAL ANEMIA

Two groups of workers have concerned themselves with the relative values of sole diets of raw or pasteurized milk in producing nutritional anemia in the rat. Scott and Erf (1) report results showing that a special milk; when fed raw, allowed a better gain in weight and greater increase in red cell count and hemoglobin than when the same milk was fed after holding pasteurization. Some commercially pasteurized milk gave inferior results to those obtained with the special pasteurized milk. It is not stated whether the rats were fed individually or in groups and what their food consumptions were. Neither are any copper and iron analyses of the milk reported. Krauss, Erb, and Washburn (2) report results contrary to those of Scott and Erf. They fed milk from the same bulk to two groups of rats, one group receiving raw milk and the other the same milk after pasteurization. The animals in both groups developed nutritional anemia as characterized by a marked decrease in hemoglobin and erythrocyte count and the decreases were at the same rate in both groups. No data are given for the food intake or growth.

## EXPERIMENTAL

*Methods.* The procedure of Elvehjem and Kemmerer (3) was followed in rendering the rats anemic. The young were weaned at three weeks, and then placed on the experimental diet of milk alone for an eight weeks period. The rats were housed in individual galvanized wire cages with  $\frac{1}{2}$  inch mesh screen bottoms. The two series of experiments conducted were similar except in the milk fed. They were carried out by the paired feeding method using males only. Both members of any one pair were litter mates and as similar as possible as to weight at the beginning of the experimental period. Those on the sole milk diet received no water. The control group consisted of litter mates fed on the stock diet and water *ad lib*.

The rats in both series were weighed weekly and food consumption rec-

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The data presented are taken in large part from the thesis of H. A. Lasby presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. All pertinent individual data are available for reference in the Division of Agricultural Biochemistry.

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ords were kept. Hemoglobin determinations were made at the time of weaning and weekly thereafter. The method used was that of Newcomer (4). Blood was obtained by cutting with a razor the tip of the tail, previously dipped in hot water to promote the flow of blood. Calcium chloride was first used to stop the flow of blood but later collodion was substituted, as it proved to be more satisfactory.

Series I. Seven pairs and three controls were used. The milk fed was obtained fresh daily, except Sunday, from the University of Minnesota Agricultural Experiment Station Dairy herd. The experiment was conducted during May, June and July when the cows were on pasture. No special containers were used for the collection of the milk. It was brought up from the barns in a tin pail from which it was transferred to glass containers for storage and pasteurization. A portion of each day's milk was fed raw to one group and a portion was pasteurized in glass and fed as such to the other. For pasteurization, the milk was placed in stoppered 1 l. Erlenmeyer flasks, which were then immersed to the neck in a water bath. A temperature of 62°-63° C was maintained in the flask for 30 minutes. Subsequently the flasks and milk were cooled as rapidly as possible in running cold water and stored, as was the raw milk, in a cold room at 40° C. No attempt was made to standardize the fat content of the milk fed. Feedings were made morning and afternoon. Definite quantities of milk were measured by pipette into glazed porcelain ramekins placed on the screen bottom of the cage. Any milk unconsumed at the next feeding was measured.

Series II. Eleven pairs and three controls were started. The milk used was commercial<sup>1</sup> raw and pasteurized milk obtained during February and March, thus representing late winter feeding. Both were obtained from the same bulk. It contained 35 per cent butter fat. The pasteurization had been carried out in Yundt-Gridley stainless steel tanks by the holding process at 143.5° F. (62° C.) for 30 minutes. Quart aliquots were taken each day and delivered for consumption the following day, except Saturday, when two quarts of Friday's raw and pasteurized milk were delivered for Saturday's and Sunday's use.

*Chemical analyses.* Analyses for iron and copper were made on the raw and pasteurized milk. In series I random samples were taken every week and analyzed. In Series II, 100 cc. aliquots were taken daily and the analyses made on weekly composites. The method used for iron was that of Elvehjem and Hart (5), Kennedy (6), and Elvehjem (7). In Series I the copper was determined by the method of Elvehjem and Lindow (8)

<sup>1</sup> This milk was furnished through the courtesy of the Franklin Cooperative Creamery Association of Minneapolis. We acknowledge our indebtedness to them for it, and especially to Ass't. Supt. Walter Ahlstrom, for insuring the taking of samples and their delivery.

as modified by Gebhardt and Sommers (9) and in Series II by the method of Cherbuliez and Ansbacher (10) and Ansbacher, Remington and Culp (11). The latter was believed to be more accurate and certainly less tedious.

**Results.** Series I. The results obtained are presented graphically in figure 1. The mean hemoglobin value for the raw milk group decreased

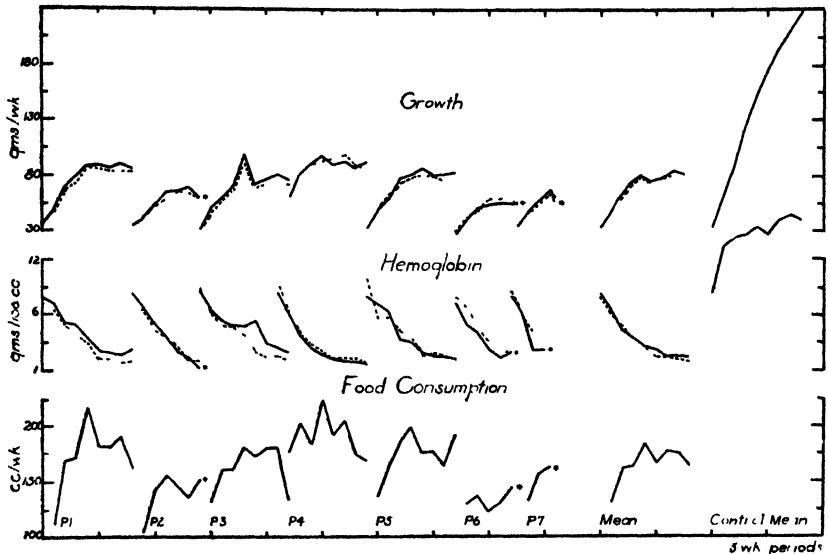


FIG. 1. Hemoglobin content of blood, body weight and milk consumption of individual rats fed raw and laboratory pasteurized summer milk from Station Dairy herd on pasture, together with mean values and the means of the blood hemoglobin and body weight of controls fed stock diet. The continuous lines represent raw milk feeding and the dotted lines pasteurized milk. The death of an animal before the end of the eight weeks period is starred.

from 7.80 to 2.31 gms. per 100 cc. while that of the pasteurized group decreased from 8.08 to 1.88 in eight weeks. The mean weight of the rats fed raw milk increased from 33.14 to 83.5 gms. in contrast to an increase from 33.71 to 78.5 gms. for those fed pasteurized milk. The data were treated statistically to test for the significance of the differences between the means of hemoglobin and the weight values obtained from the two groups of rats.

Using Fischer's application of 'Students' distribution of  $t$  and the 5 per cent level of significance the data in table 1 show that the differences for each successive weekly interval were not significant except for the fifth week hemoglobin values. This cannot be considered important inasmuch

TABLE 1

*Statistical significance of weekly differences in mean hemoglobin content of blood and body weight of rats fed raw and pasteurized milk in paired feeding tests*

CALCULATIONS FROM HEMOGLOBIN			CALCULATIONS FROM BODY WEIGHT	
<i>Week</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
0	1.9277	0.0780	0.8738	0.4018
1	1.4279	.1786	0.8356	.4228
2	1.0463	.3194	0.3999	.6962
3	0.8717	.4020	1.5515	.1582
4	0.3036	.7704	0.5104	.6212
5	3.4662	.0062	0.4004	.6976
6	0.5587	.5914	0.1887	.8542
7	1.3859	.1086	2.0751	.0914
8	1.9765	.0966	1.7363	.1320

as no statistically significant difference occurred in either the preceding or succeeding week

The copper and iron analyses of the raw and pasteurized milk gave results indicating that the same quantity of metals was present in each. Six iron analyses on raw milk made during the eight weeks period varied from 1.23 to 1.48 mgms. per liter with a mean of 1.35 and on the same milk after pasteurization from 1.20 to 1.48 mgms. per liter with a mean of 1.35. Six copper analyses made on the same milk varied from 0.11 to 0.17 mgms. per liter with a mean of 0.14 while those on the pasteurized milk varied from 0.12 to 0.16 mgms. per liter with a mean of 0.14 mgm.

Series II. Since the conditions used in laboratory pasteurization are not those under which market milk is treated, a series was run in order to determine whether commercial pasteurization had any effect on the severity of the anemia produced by feeding it as the sole diet to rats.

The results obtained are plotted in Figure 2. Pair number eight was omitted from all calculations because that member of the pair being fed pasteurized milk escaped from the cage one night and there was a subsequent rise in hemoglobin.

It may be seen that there was a much wider spread between the hemoglobin and growth curves of the two rats of a pair than in those of Series I. In all cases that member of a pair receiving pasteurized milk grew better and had more hemoglobin. If the rat on raw milk had a higher hemoglobin or weighed more at the beginning of the experiment, there was a subsequently poorer growth or a greater decrease in hemoglobins than on pasteurized milk, resulting in lower values at the end of the experiment. In all cases the milk consumption was controlled by that member of the pair receiving raw milk.

Table 2 shows the statistical significance of the weekly differences of the mean values. It is seen that the differences in the mean hemoglobin values

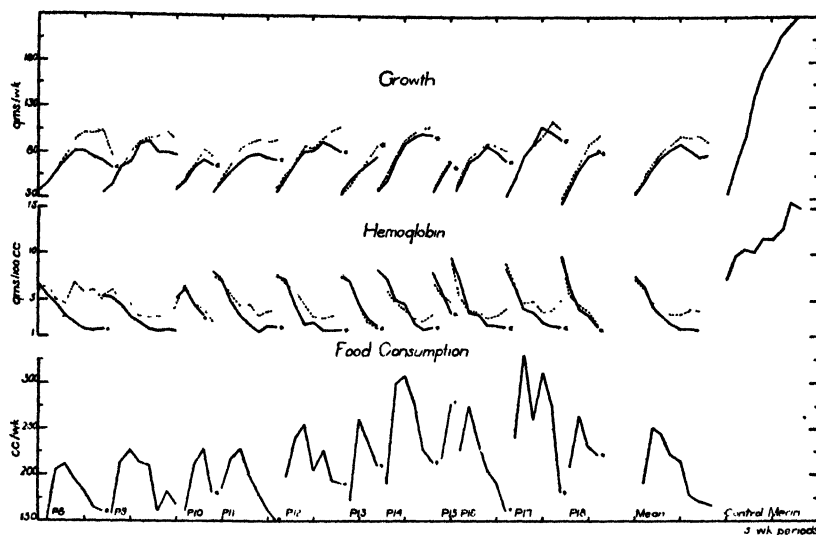


FIG. 2. Hemoglobin content of blood, body weight and milk consumption of individual rats fed commercially raw and pasteurized milk from winter feeding, together with mean values and the means of blood hemoglobin and body weight of controls fed stock diet. The continuous lines represent raw milk feeding, and the dotted lines pasteurized milk. The death of an animal before the end of the eight weeks period is starred.

were statistically significant at the end of the second week and thereafter to the end of the experiment. The fact that the differences of the first two weeks were highly insignificant does not mitigate against the significance found subsequently. A lapse of time before the appearance of the difference would be expected since the difference in the concentrations of copper and iron as shown by chemical analyses were small. When the mean weekly

TABLE 2

*Statistical significance of weekly differences in mean hemoglobin content of blood and body weight of rats fed commercial raw and pasteurized milk in paired feeding tests*

CALCULATIONS FROM HEMOGLOBIN			CALCULATIONS FROM BODY WEIGHT	
Week	t	P	t	P
0	0.2822	0.7828	0.3419	0.8134
1	0.7104	.4866	0.9687	.3456
2	2.0342	.0576	0.6187	.5434
3	2.4231	.0284	2.0437	.0572
4	2.8503	.0130	1.0779	.2966
5	6.8123	.0000	1.2947	.2264
6	3.1061	.0112	0.5347	.6080
7	4.7452	.0090	3.7983	.0194

weights of the groups are considered, it may be seen that those fed pasteurized milk had consistently greater mean weights than those fed the raw milk. However, Table 2 shows that the difference was significant only in the third and seventh weeks. The significance in the third week was border line and since that in the seventh was based on only four individuals it is doubtful that it should be considered as truly significant. The greater differences observed in this series are no doubt due to the iron and copper intakes of the rats fed the pasteurized milk. However, it seems that although this greater intake was sufficient to produce significant differences in the hemoglobin values, slightly more would be necessary to produce equally significant differences in growth.

Chemical analyses of the commercial pasteurized milk showed that it differed in copper and iron content from the raw milk. Eight analyses for iron made during the eight weeks period showed for raw milk a range from 0.85 to 1.49 mgms. per liter with a mean of 1.13 while for pasteurized milk the range was from 1.06 to 1.64 mgms. per liter with a mean of 1.34. When the same milk was analyzed for copper, the raw milk showed a range from 0.15 to 0.24 mgms. per liter with a mean of 0.19 and the pasteurized milk ranged from a content of 0.23 to 0.32 mgms. per liter with a mean of 0.27. It seems evident that the milk picked up copper and iron in the milk plant although the stainless steel pasteurizing tanks could not have been responsible. It is this additional quantity of the metals in the pasteurized milk which is reflected in the higher hemoglobin and greater growth of the rats fed the commercially pasteurized milk.

As the experimental period progressed all rats in both series developed an appearance characteristic of nutritional anemia. In case of piebald rats the black fur became gray except in small area on the head. After the rats had been on the milk diet for some time it was noted that the incisor teeth were growing out without the characteristic brown pigment on the outer surfaces. In some the dark coloration was still visible on the distal ends of the teeth. This lack of tooth pigment seems to be a characteristic result of milk diets. Gross autopsies performed revealed that all organs and muscle tissue were bloodless in appearance. The caecum was almost invariably enlarged and often the heart. In Series I no difference could be noted in the appearance and behavior of those fed raw milk and those fed pasteurized milk. In Series II, however, there was a marked difference in the dispositions of the two groups. Those fed pasteurized milk were more active and eager for food, while the others tended to be inactive and huddled in the corners of the cages. In all cases the animals fed raw milk controlled the food consumption of the pair. The mortality in this series was much greater than in Series I. Of the eleven pairs started, only one survived the eight weeks experimental period. In the ten pairs in which early death occurred, nine of the ten rats dying were fed raw milk. Why the mortality

in this series was higher than in Series I is uncertain. However, it may have been due both to a smaller fat content if the commercial milk which was standardized to 3.5 per cent butter fat content, resulting in a lower caloric intake than in Series I, and to the fact that this milk which was secured for study in the latter part of the winter had a lower vitamin content, particularly vitamin A, as the result of the type of winter feeding practiced on the farms where the milk was produced.

## PART II. CALCIUM, PHOSPHORUS, AND NITROGEN METABOLISM

That the heat treatment of milk alters certain of the milk salts is now definitely established. Whether this change alters their nutritive value is still unanswered. Considerable experimental nutritional work has been done on the comparison of the retention of the calcium, phosphorus, and nitrogen of raw and pasteurized milk. Most of the results are inconclusive and few can pass uncriticized. For an accurate comparison the raw and pasteurized milk fed must come from the same bulk and a sufficient number of animals must be used to insure the required degree of accuracy in the subsequent evaluation of the results. The effect of other articles of diet on the retention of these minerals is not known. There also appears to be species differences so that results obtained from one species are not necessarily applicable to another.

Daniels, Stuessy, and Francis (12) compared the growth of rats fed milk which had undergone various heat treatments. Ellis and Mitchell (13) carried out carefully controlled calcium balance studies with rats fed raw or pasteurized milk plus a basal ration containing a small amount of calcium. They found that the animals fed raw milk gained more in weight and showed a higher calcium content of the carcass than those fed pasteurized milk. In both cases the results were statistically significant. However, when the average calcium retention was as high as 98 per cent for rats on raw milk and 92 per cent for rats on pasteurized milk the calcium intake was probably too low to be of any practical importance.

Krauss, Erb, and Washburn (2) measured the ability of rats to use calcium and phosphorus from raw pasteurized milk by analyzing the femurs of rats after a period of feeding on the milk as a sole diet. No differences in the percentage of ash, calcium and phosphorus were found.

## EXPERIMENTAL

**Methods.** Male rats 10 weeks of age, and weighing from 225 to 292 gms. were selected from an inbred strain selected for high efficiency of food utilization during growth. At this age the rats selected were still storing calcium, their normal growth curve was still a straight line, and their efficiency was known. The paired feeding method was used. Male litter mates were paired as nearly as possible for weight. The study included

six pairs from two litters. One member of each pair was fed raw milk and the other pasteurized milk from the same bulk. The milk fed was from the University of Minnesota Agricultural Experiment Station herd. The experiment was conducted in late September and early October. The method of pasteurizing was the same as that used in Series I of the anemia studies. Seven day collection periods were used. The rat was always placed on the particular diet to be used three days before collections were begun. Three successive collection periods were used for each pair. The order of feeding for one member of a pair was raw, pasteurized, and raw, and for the other pasteurized, raw, and pasteurized. This alternation was used in order to minimize the possible influence of the order of feeding the two milks. The milk was fed twice a day, morning and late afternoon. To the morning feeding there was added 1 cc. of a slightly acidulated solution of ferric chloride and copper sulfate made up so that 1 cc. contained 0.4 mgms. of Fe and 0.32 mgms. of Cu in order to prevent nutritional anemia. The milk thus supplemented was completely consumed by both members of a pair before more was fed. The milk was measured out by a pipette and any unconsumed after 24 hours was measured.

The cages used were especially designed for metabolism studies. The rats were weighed weekly and food consumption records kept. The urine and feces were collected after each period and analyzed for calcium, phosphorus, and nitrogen.

Similar analyses were made on aliquots of weekly composite samples of the raw and pasteurized milk fed. The milk was preserved with formaldehyde until the analyses were made. Fifteen pairs of balance determinations were made using six pairs of rats. By subtracting the total amount of a mineral excreted in the urine and feces from the intake as supplied by the milk, the balance was obtained. For the sake of comparison, the balance values were then calculated on the basis of the percentage of the intake retained. In order to compare the percentages of the intake of the respective minerals which were retained on the diets of raw and pasteurized milk, the mean values were calculated.

*Chemical analyses.* The calcium and phosphorus determinations were made on the samples by the method of Morris, Nelson, and Palmer (14). The nitrogen was determined by semi-micro Kjeldahl method of Cavett (15).

*Results.* The results obtained are presented in Table 3. The significance of the difference was then determined as in Part I.

The mean percentages of intake retained together with the corresponding probabilities are given in Table 4.

There was no significant difference between the retentions on the diets of raw and pasteurized milk. The slight differences which were found favored the pasteurized milk.

TABLE 3  
*Calcium, phosphorus and nitrogen balances*

PAIR NO.	BAT NO.	DIET	Ca		P		TOTAL P		P		N		TOTAL N		N		
			INTAKE	BALANCE	INTAKE	BALANCE	EXCRETION	INTAKE	BALANCE	INTAKE	BALANCE	EXCRETION	BALANCE	INTAKE	BALANCE		
			mgm.	mgm.	percent	percent	mgm.	mgm.	percent	percent	mgm.	mgm.	mgm.	mgm.	percent	percent	
1.	1728	1. raw	539.8	195.4	63.8	435.7	250.9	184.8	57.6	2633.8	2141.8	492.0	81.3				
	1730	1. past.	531.7	394.4	137.3	423.4	267.6	155.8	63.2	2553.6	1814.6	737.2	71.1				
	1729	1. raw	363.2	262.8	120.4	302.9	212.1	90.8	70.1	1995.5	1531.9	463.6	78.1				
	1731	2. past.	631.8	478.5	153.3	477.9	287.2	190.7	60.1	3068.0	2470.2	597.8	80.5				
	1731	1. past.	371.0	239.2	131.8	291.3	192.6	98.7	66.1	1807.5	1519.1	288.4	84.1				
	2. raw	625.6	478.7	146.9	76.5	456.2	280.3	205.9	57.7	2984.8	2268.7	716.1	76.0				
3.	1733	1. raw	532.7	300.1	232.6	56.4	429.9	273.2	156.7	63.6	2598.8	2756.1	842.7	67.6			
	2. past.	637.9	378.8	259.1	59.4	486.2	221.5	261.0	45.9	3097.5	1960.9	1136.6	63.3				
	3. raw	609.7	450.1	159.6	73.8	465.9	270.4	195.5	58.1	2930.1	2255.0	675.1	77.0				
4.	1735	1. past.	537.7	297.1	140.6	428.1	257.7	170.4	60.2	2582.1	1833.2	748.9	71.0				
	2. raw	631.6	354.0	267.6	57.6	490.9	234.4	256.5	47.8	3013.5	2358.8	654.7	78.3				
	3. past.	620.1	385.0	235.1	62.1	458.5	244.6	213.9	53.4	2985.3	2414.3	571.0	80.9				
5.	1766	1. raw	625.0	393.8	231.2	413.9	239.8	174.1	57.9	2391.4	1852.1	539.3	77.5				
	2. past.	604.5	487.9	116.6	80.7	464.7	276.1	188.6	59.4								
	3. raw	603.8	490.4	113.4	81.2	474.6	264.7	209.9	55.8	2376.0	1725.0	651.0	72.6				
	1767	1. past.	576.3	267.6	308.7	46.4	298.7	241.5	157.2	60.6							
	2. raw	624.4	436.5	187.9	69.9	445.6	252.9	192.7	56.8								
	3. past.	622.1	453.1	169.0	72.7	457.0	254.5	202.5	55.7								
6.	1765	1. raw	580.8	333.4	247.4	57.4	384.6	224.7	159.9	58.4	2922.2	1775.9	446.3	79.9			
	2. past.	612.3	422.2	190.1	69.0	470.7	267.8	202.9	56.9	2593.5	2235.1	358.4	86.2				
	3. raw	639.8	479.4	160.4	74.9	502.9	286.1	216.8	56.9	2756.7	2304.9	451.8	83.6				
7.	1764	1. past.	561.6	318.9	242.7	56.8	388.6	191.2	197.4	75.0	2231.3	1673.7	557.6	75.0			
	2. raw	628.4	483.0	145.4	76.9	448.4	286.0	162.4	81.3	2650.5	2150.9	499.6	81.3				
	3. past.	643.1	466.4	176.7	72.5	472.4	298.2	174.2	83.0	2675.6	2313.3	374.3	83.0				
8.	1767	1. raw	498.0	335.2	162.8	329.8	211.5	118.3	73.0	1905.5	1591.0	314.5	73.0				
	2. past.	483.4	346.1	137.3	371.6	291.4	110.2	69.8	75.5	2047.5	1429.8	617.7	69.8				
	3. raw	538.5	491.2	147.3	72.7	423.3	240.6	182.7	75.5	2920.2	1751.1	569.1	75.5				
9.	1769	1. past.	382.8	267.9	114.9	70.0	264.9	214.7	50.2	1578.5	1417.8	160.7	89.8				
	2. raw	480.2	329.2	128.0	73.3	342.7	225.6	117.1	58.8	2025.4	1190.9	834.5	58.8				
	3. past.	546.6	409.4	137.2	74.9	401.5	252.3	149.2	80.7	2269.4	1912.9	256.5	80.7				

## BONE ANALYSES

*Methods.* For the purpose of determining whether the calcium and phosphorus of raw and pasteurized milk were equally available for bone formation, analyses were made on bones of the rats used in Series II of the anemia studies. The femurs and tibias were removed immediately after death, stripped of adhering flesh, and stored until the analyses were made.

TABLE 4

*Mean percentage retention of calcium, phosphorus and nitrogen from raw and pasteurized milk and P value of differences*

ELEMENT	RETENTION ON RAW MILK	RETENTION ON PASTEURIZED MILK	P
	<i>per cent</i>	<i>per cent</i>	
Calcium	68.9	68.3	0.6009
Phosphorus	58.5	60.5	0.1120
Nitrogen	76.0	77.5	0.1616

At that time the femur and tibia of one leg from each animal were selected, crushed in filter paper, and extracted with alcohol for 48 hours and with ether for 24 hours. The percentage of ash in the bones was determined and the ash analyzed for calcium and phosphorus according to the method of Morris, Nelson, and Palmer (14). Weekly aliquots of the milk were also analyzed for calcium and phosphorus by the same method. The mean calcium values of the weekly aliquots of raw and pasteurized milk were 11.96 mgm. and 12.01 mgm. per liter respectively. The corresponding phosphorus values were 8.92 mgm. and 8.96 mgm. per liter of raw and pasteurized milk.

*Results.* Results of the bone analyses of the eleven pairs of rats are presented in Table 5.

These data were treated statistically by the method previously referred to. A probability of 0.054 was obtained that the percentage of ash in the bones of rats fed raw milk was significantly different from that of the rats fed pasteurized milk. This is a border line value. The percentage of ash was then correlated with the age of the rat, since the age might influence the percentage of ash and the rats used were of different ages depending on the time during the experimental period at which death occurred. A correlation coefficient equal to + 0.7638 was obtained but this was not significant for the number of samples correlated. Since the femur and tibia of the other hind leg of the rats were also available, the length of these was obtained by means of calipers. The means of these data also are shown in Table 5. The total length of the two bones was then correlated with the percentage of ash. This was done because the rats fed pasteurized milk grew better but their bones showed a lower ash content than did the bones

TABLE 5

*Calcium and phosphorus content and length dry-extracted femur plus tibia of rats dying from anemia after consuming commercial raw and pasteurized milk*

MILK	BONE COMPOSITION			TOTAL LENGTH FEMUR PLUS TIBIA
	Total Ash	Ca	P	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>mm.</i>
Raw	58.93	22.13	9.38	5.326
Pasteurized	56.99	21.67	9.16	5.389

of the rats fed raw milk. Hence, if a longer bone showed a lower ash content, it should contain more organic and less mineral matter. The correlation coefficient between the length of the leg bones and the ash content in the raw group was + 0.6238 and in the pasteurized group + 0.2493, neither of which are significant. It seems, therefore that the difference in the mean percentage of ash in the bones of the two groups is probably not significant.

#### SUMMARY AND CONCLUSIONS

Experiments designed to compare the severity of nutritional anemia developed in comparative groups of rats when one group was fed raw milk and the other the same milk after pasteurization have been carried out. The results of the experiment show:

1. When the pasteurization of milk is carried out in glass in the laboratory, the severity of the anemia and the growth of the rats are not significantly different on diets of raw or pasteurized milk.

2. The iron and copper contents of milk are not affected by pasteurization in glass in the laboratory.

3. When the pasteurization of milk is carried out commercially, the anemia developed may be less severe than on raw milk and the growth better.

4. The iron and copper contents of milk commercially pasteurized may be greater than that of the milk before the heat treatment due to contact with the metallic equipment in use in commercial milk plants.

Experiments designed to compare the calcium, phosphorus, and nitrogen balance metabolism of paired rats, one of which received a sole diet of raw milk and the other a sole diet of the same milk after holding pasteurization in glass in the laboratory, have been carried out. The results of these experiments lead to the following conclusions:

1. The calcium retentions are the same on the two diets.

2. The phosphorus retentions are slightly but not significantly greater on pasteurized milk.

3. The nitrogen retentions are slightly but not significantly greater on pasteurized milk.

Analyses of the percentage of ash, calcium and phosphorus have been made on dry-extracted femur-tibia bones of rats fed a sole diet of raw or commercially pasteurized milk.

The following results were found:

1. The bones of the rats fed raw milk had a higher percentage of ash than those of the rats fed pasteurized milk. The difference was probably not significant.

2. The mean calcium and phosphorus contents of the bones of the rats fed raw milk were slightly but not significantly higher than those of the rats fed pasteurized milk. The ash, calcium, and phosphorus differences while not significant may have been due, in part, to the better growth of the rats fed the pasteurized milk.

3. The mean calcium and phosphorus contents of the milk before and after pasteurization were the same.

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# American Dairy Science Association Announcements

## ANNUAL MEETING AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY FARM, ST. PAUL

June 24 to 27, 1935

### GENERAL INFORMATION

There has been a slight change in the general plan of the meeting from that announced in the February issue of this Journal. It is now planned to have the opening session Monday evening, June 24th, at which there will be the usual addresses of welcome and opportunity to transact such matters of business as the officers desire to present to the Association. A second and concluding business session will be held Wednesday afternoon, June 26th. The opening session will be followed by a general get-together in the Dormitory Parlors where it is expected that most of the members will have secured rooms.

Plans are taking shape for the opening scientific session. Tuesday morning, June 25th. This is to be a symposium entitled "Improving the Germ Plasm of Domestic Plants and Animals" which will be held jointly under the auspices of Section O. A. A. S., the American Dairy Science Association, the Corn Belt Section of the American Society of Agronomy, the American Society of Horticultural Science Great Plains Section, the American Society of Plant Physiologists, and possibly also the American Phytopathological Society. Our own Association will be represented on this symposium by Dr. Jay L. Lush, of Iowa State College. The program committee hopes that this unusual opportunity to meet with our colleagues and friends in other fields of agricultural science will prove to be the special feature of the 1935 meeting which will long remain a pleasant memory as well as be an inspiration to all of us.

### SECOND CALL FOR PAPERS AND ABSTRACTS

Members are again invited to send titles of papers for the Sectional Scientific sessions. Each title should be accompanied by an abstract of 300 words or less. *All communications relative to papers should be sent directly to the chairman of the Program Committee* All titles and abstracts must be received by May 1st in order that the program may be arranged and printed in the June issue of this Journal and the abstracts be made available in printed form for the meeting. Authors are asked to indicate the

section before which they wish to appear. Authors who submit titles and abstracts and later learn that they will not be able to attend the meeting are urged to advise the chairman if possible by May 10th in order that the paper may be placed at the end of the program for presentation by vote of the membership present.

L. S. PALMER, *Chairman*  
Program Committee  
University Farm, St. Paul

# JOURNAL OF DAIRY SCIENCE

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## A STUDY OF THE INFLUENCE OF THYROXIN ON MILK SECRETION\*

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A knowledge of the physiological factors affecting milk secretion is essential to the study of the causes of variation in milk yield among dairy cows. It is reasonable to suppose that the activity of the mammary gland is a direct function of the general metabolism of the animal. If this supposition is correct, those factors that affect the metabolism should have a similar influence on milk yield. The stimulating effect of thyroxin and thyroid extracts upon the metabolic rate of animals has been well established (1, 10, 11). Henderson (9) has studied the effects of various stimulants and galactagogues over two day test periods. McCandlish (13, 14) studied the effect of drug administration on milk yield in a two day period. Ott and Scott (15, 16) injected animal extracts and observed the number of drops of milk secreted in a five minute period following injection. Changes brought about by the various treatments were not significantly large or of long enough duration that the data could be applied to the study of the problem of milk secretion.

Turner (19) has shown that galactin, a hormone elaborated in the anterior pituitary gland, is able to initiate milk secretion in the developed mammary gland. Corner (2) has shown the positive effects of hypophyseal extracts in the hormonal control of lactation. Gowen and Tobey (5) found that injections of insulin and phloridzin caused a marked decline in milk yield. Grimmer and Wentzel (8) claim that thyroidectomized goats secreted milk at a rate comparable to that before thyroidectomy. Graham (6) has studied the effect of thyroidectomy and thyroid feeding on milk secretion and milk fat production of cows. He shows that thyroidectomy and a similar control operation without the thyroidectomy caused a rapid decline in milk secretion and that the effects of the thyroidectomy could not be distinguished from those of the control operation. Thyroid feeding to thyroidectomized cows brought the milk yield level back to that before the operation. Thyroid feeding to normal cows caused a rise in

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milk flow when the rate of lactation was falling but was ineffective when the curve was rising or at its peak.

The study here presented was designed to determine the effect of intravenous thyroxin injections upon the quantity of milk secreted by dairy cows and upon the composition of the milk.

#### EXPERIMENTAL PROCEDURE

Four cows were injected intravenously with thyroxin. Two purebred Brown Swiss cows, #1559 and #1562, were used to study the effect of the injections on the composition and quantity of milk secreted. A veterinary physical examination in conjunction with the Brom-thymol-blue test, the cell count, and the chloride content of the milk showed the animals to be free from mastitis which might alter the composition of the milk. #1562 was injected with 25 mgm. of thyroxin and after a 10 day interval she was given daily injections of one mgm. for 18 days. Then #1559 was given identical treatment for 28 days. #1562 was then given a second 28 day period of treatment.

The other cows used were purebred Holstein-Friesians. #1525 in declining production and the other, #1400, at the peak of her production. They were given 25 mg. injections at seven day intervals for three weeks to study the effect on the quantity of milk secreted.

The following constituents were determined in the milk of the first two cows; Specific gravity with a lactometer, fat percentage by the Babcock method, solids-not-fat calculated by the equation  $\% \text{ SNF} = 1.4 + .2F + .14$ , lactose by the colorimetric method of Folin and Wu (4) for blood sugar and modified for lactose by Owen and Gregg (17), total nitrogen by the Kjeldahl method and protein calculated as  $\% \text{ Protein} = N \times 6.38$ , and lactalbumin and lactoglobulin by the colorimetric method of Greenberg (6) for serum proteins. Analyses were made every second or third day on a sample representing an aliquot portion of each milking during the 24 hours.

Body weights were taken on these animals at the beginning and end of each 28 day period. Weighings were made three days in succession at the same hour each day and the three weights averaged to secure the weight for the period.

Data given by Thompson, Thompson, and Dickie (18) show that one tenth of a milligram of Squibb's thyroxin will increase the basal metabolic rate of a person of 140 pounds weight by 10%. On a comparable weight basis one milligram should increase the basal metabolic rate of a 1400 pound cow by 10% and 25 mm. per week should increase the basal metabolic rate about 30%.

#### EXPERIMENTAL DATA

The accompanying graphs (Figures 1, 2, 3, 4) show the data obtained for daily milk weights, milk fat percentage, pounds of milk fat produced,

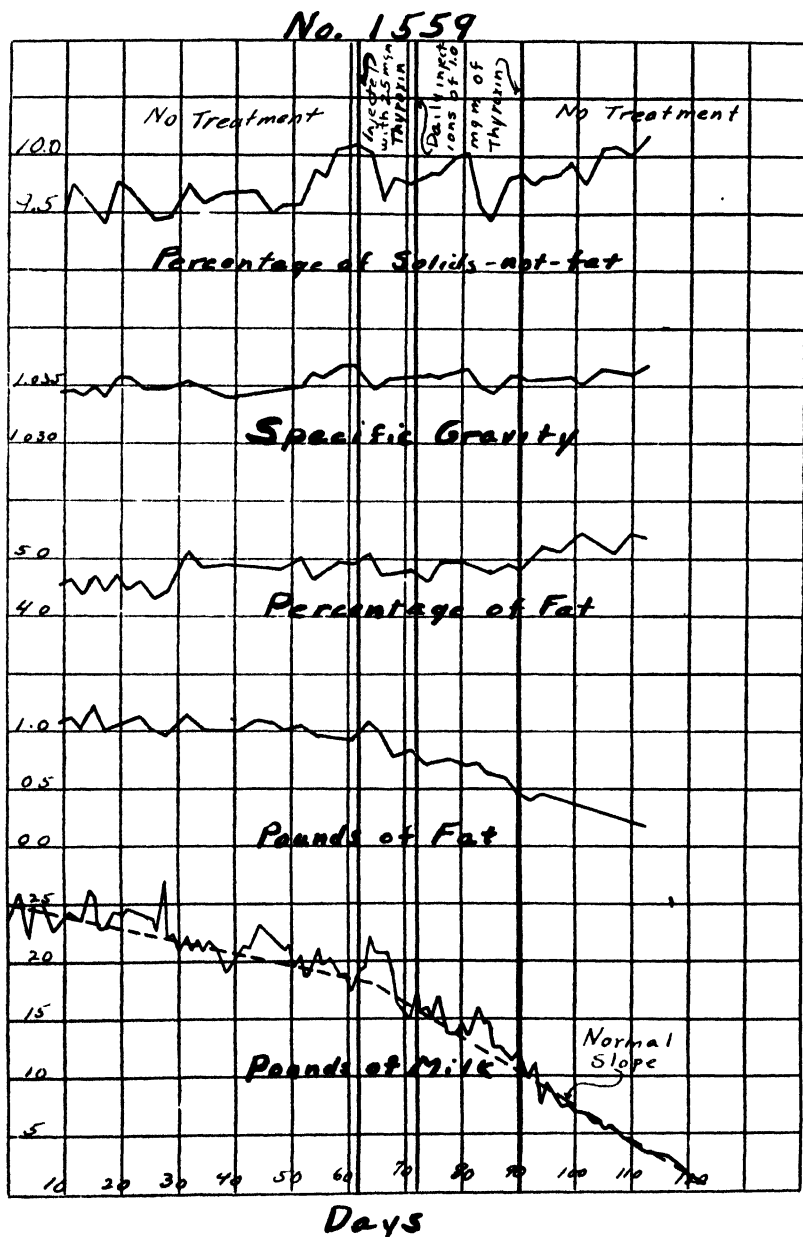


FIG. 1. Graph showing daily production of milk, milk fat, fat percentage, specific gravity, and solids-not-fat for No. 1559.

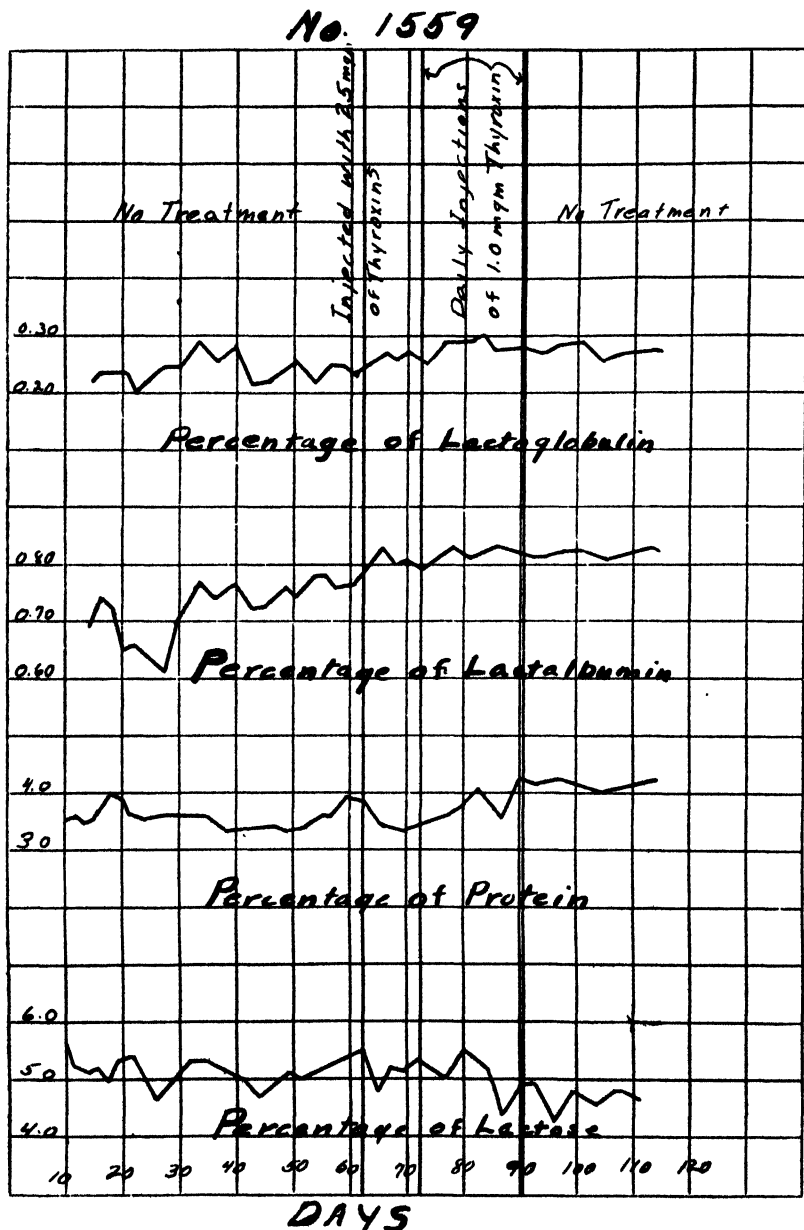


FIG. 2. Graph showing percentages of lactose, protein, lactalbumin, and lactoglobulin for No. 1559.

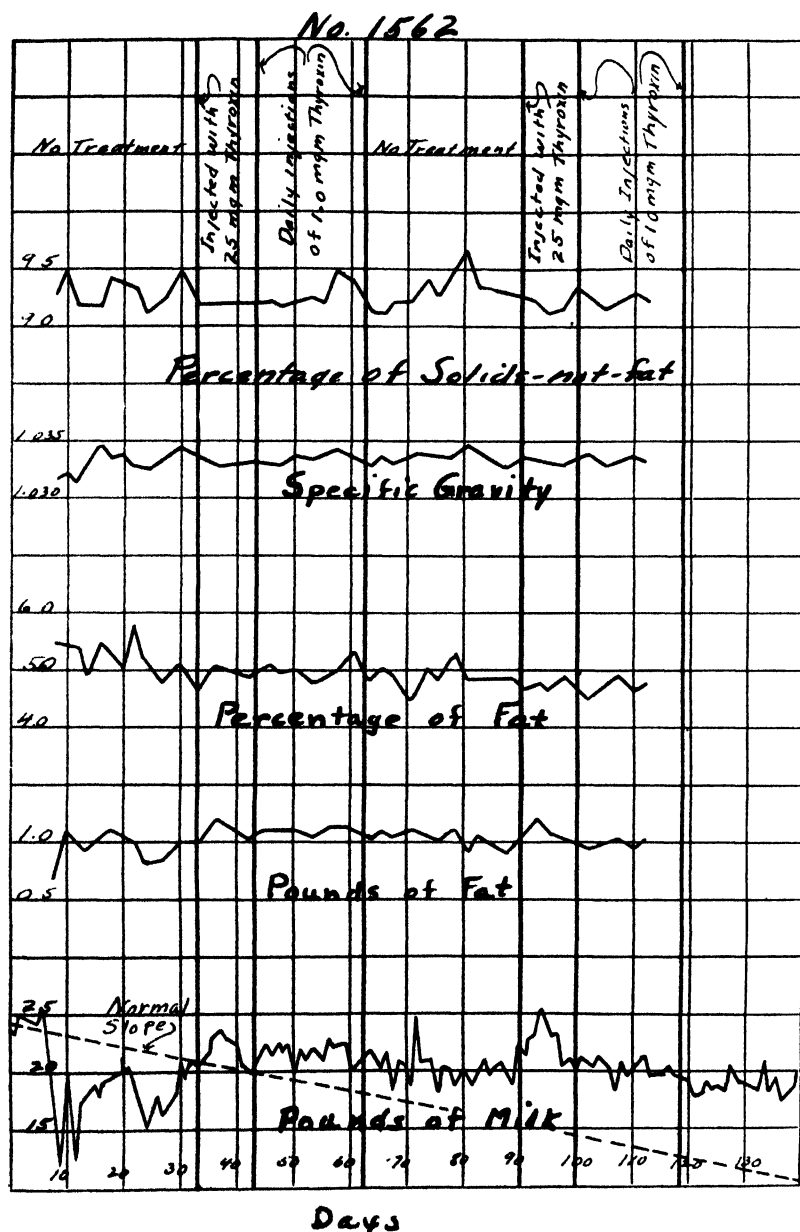


FIG. 3. Graph showing daily production of milk, milk fat, fat percentage, specific gravity, and solids-not-fat for No. 1562.

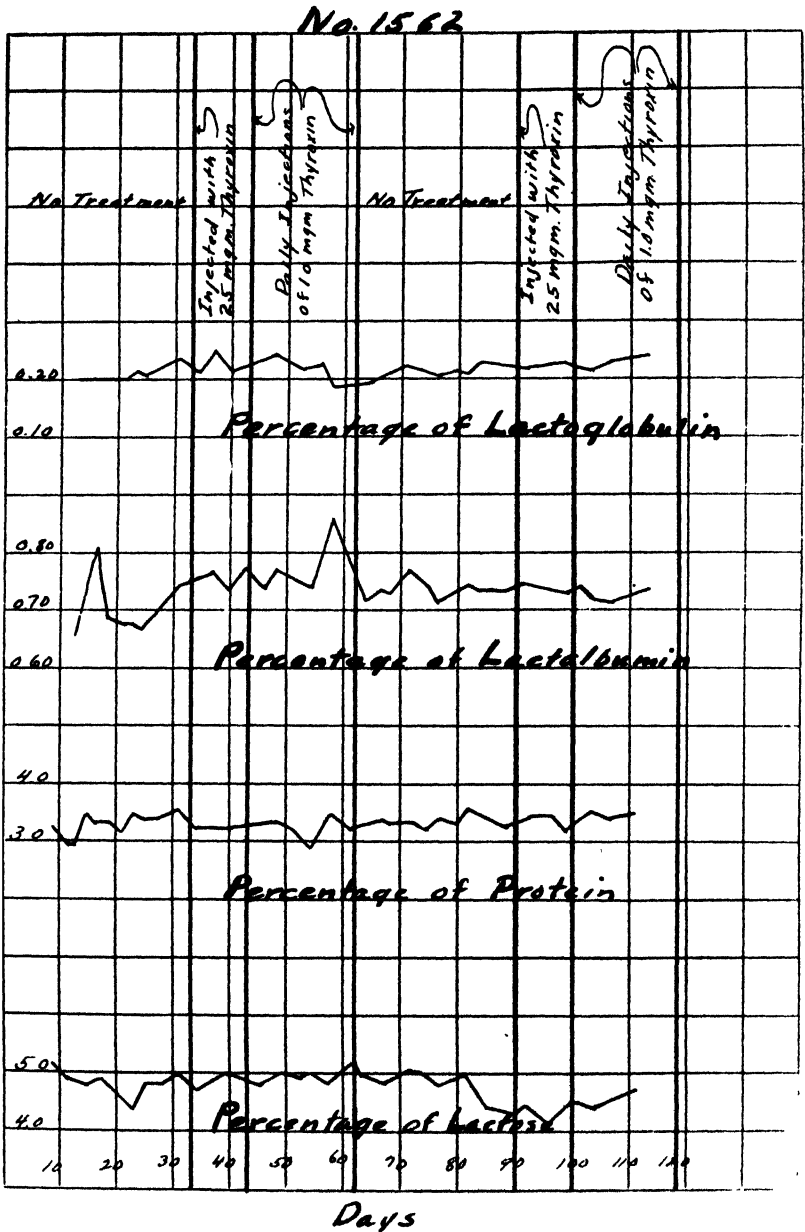


FIG. 4. Graph showing percentages of lactose, protein, lactalbumin lactoglobulin for No. 1562.

lactose percentage, percentage of protein, lactalbumin and lactoglobulin percentages, specific gravity, and percentage of solids-not-fat for cows #1559 and #1562 used in the first part of the experiment.

The following table shows the average daily production of milk, fat percentage, pounds of fat produced by weekly periods and the body weights of the animals, #1562 and #1559, by 28 day periods.

No. 1562				
<i>Week</i>	<i>Lbs. Milk</i>	<i>% Fat</i>	<i>Lbs. Fat</i>	<i>Weight</i>
12/18	20.4	5.05	1.05	1215
25	17.7	5.60	0.99	
1/1	18.3	5.25	0.96	
1/8	20.2	4.55	0.93	
15	22.2	5.10	1.14	1210
22	21.2	4.85	1.03	
29	21.5	5.00	1.07	
2/5	21.8	4.90	1.07	
12	20.7	5.05	1.04	1235
19	21.0	4.85	1.02	
26	19.4	5.00	0.97	
3/5	19.9	4.80	0.95	
12	23.2	4.75	1.10	1265
19	20.6	4.65	0.96	
26	20.2	4.80	0.97	
4/2	20.3	4.75	0.97	
No. 1559				
12/18	23.9	4.55	1.04	1175
25	23.9	4.60	1.05	
1/1	23.8	4.60	1.03	
8	22.4	4.70	1.01	
15	20.5	4.70	0.96	1220
22	21.5	4.80	1.03	
29	20.0	4.90	0.98	
2/5	19.2	4.80	0.92	
12	20.1	4.55	0.92	1225
19	15.7	4.60	0.72	
26	14.3	4.75	0.68	
3/5	13.3	4.50	0.60	
3/12	9.7	4.15	0.40	1290
19	7.1	4.95	0.35	
26	5.1	4.90	0.25	
4/2	3.1	4.90	0.15	

No. 1559 was dropped to twice daily milking on March 13 and was turned dry early in April because she was due to freshen about June 1. This fact accounts for the rapid decline in her milk yield. All other cows were milked three times daily and were handled in the same manner as the rest of the herd.

The graph (Fig. 5) shows the daily rate of milk secretion of the two

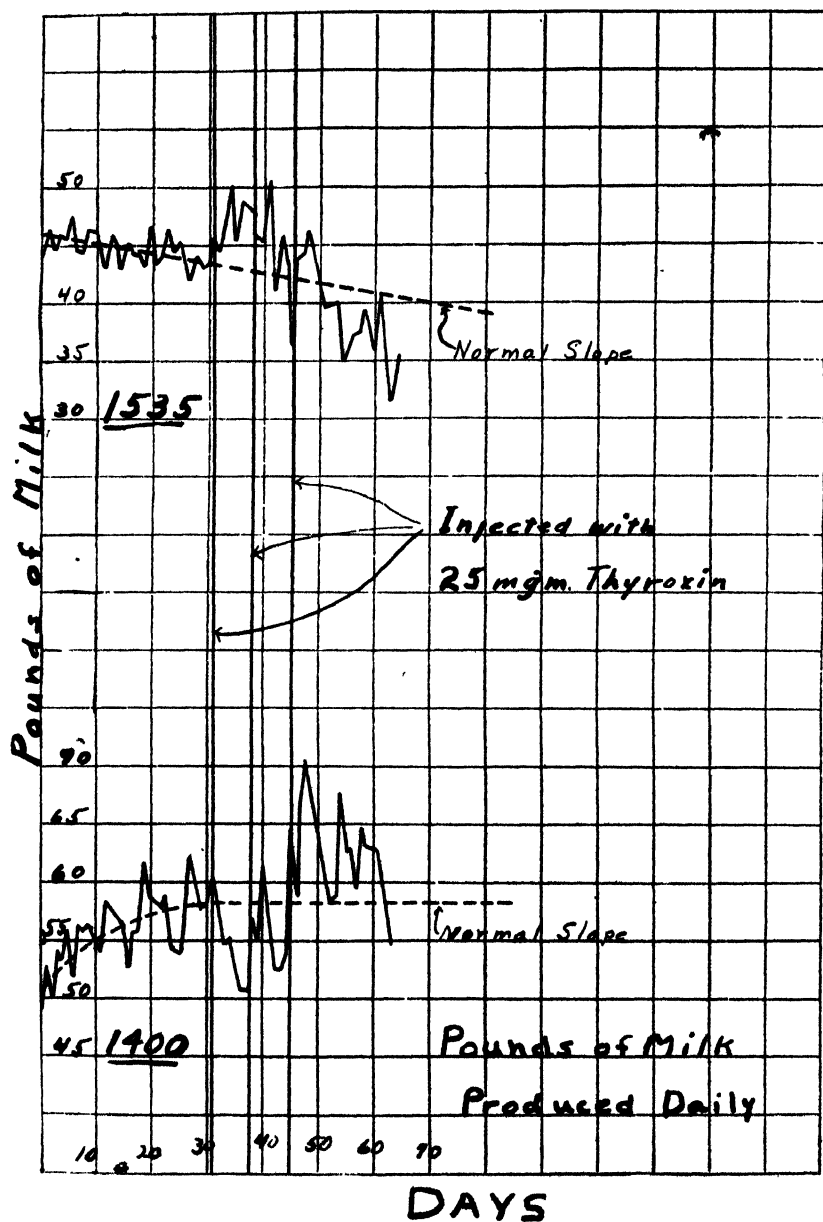


FIG 5. Graph showing daily milk production for No. 1400 and No. 1535.

cows, No. 1535 and No. 1400, used in the second part of the experiment to study the effects on milk yield of weekly injections of 25 mgm. of thyroxin. No. 1400 had been in milk 42 days and apparently was at the peak of her production when the first injection was made. She struggled so violently during the first two injections that a portion was spilled. It is estimated that she received about half of the amount of these injections and the full amount at the third dose. No. 1535 had been in milk six and one half months at the time of the first injection.

#### DISCUSSION OF RESULTS

The data show that the composition of the milk is not significantly altered by injections of thyroxin. The variations that occur are for the most part no greater than are to be expected, and do not appear to be correlated with the course of the thyroxin injections.

Loeper, LaMaire, and Tonnet (12) have found that in human beings the albumin to globulin ratio in blood plasma normally is 3:2 and that in hyperthyroidism it increases to 9:1, the change being due to an increase in albumin at the expense of the globulin fraction.

It was thought that thyroxin injections possibly would bring about a similar change in these protein fractions in the blood stream, and, provided there is a quantitative exchange of globulin between the blood and the milk in the secretion of this constituent, reflect itself in a lower lactoglobulin value since lactoglobulin is known to be identical with seroglobulin. Such a change did not appear in the milk. Unfortunately blood analyses were not made and so it cannot be said whether or not any change occurred in the blood composition. Such data would have enabled us to speculate with more certainty upon the mode of lactoglobulin secretion.

The most significant change noted was the increase in milk yield in all cases following the administration of thyroxin. In the case of No. 1562 the dosage calculated to be equivalent to a 10% increase in the basal metabolic rate resulted in an increase in milk yield of 11.2%. The increase in yield of No. 1535 resulting from administrations equivalent to a 30% increase in the basal metabolic rate was 6.5%. The two partial injections in No. 1400 caused considerable fluctuation in milk yield with the average somewhat lower than in the two previous weeks. The third complete injection brought about an increase of 9%. The rapid decline in yield of No. 1559 due to the nearness to the end of the lactation period makes a similar computation impossible in her case. With No. 1559 and No. 1562 the 25 mgm. injections were most effective in increasing milk yield and in all four animals so treated the effects were gone at the end of seven to nine days after the injections. The effects of the one milligram injections seemed to disappear as soon as the injections were stopped.

Three different stages in the lactation period are represented by the cows used in this work. The peak of production is represented by No. 1400; she showed a lower milk yield on the two partial doses, but showed an increase following the third dose. This does not substantiate Graham's (6) observations where desiccated thyroid was fed at the peak of production. The period of declining production is represented by No. 1535 and by No. 1562. In both cases the administration of thyroxin was decidedly effective in increasing milk yield. The administration of thyroxin was not particularly effective in increasing milk yield in the case of No. 1559 at the end of the lactation period. This fact indicates that the forces tending to inhibit milk flow at this time are more effective than the stimulating effect of the treatment. In all cases the effects of the administration ceased in a very short time after the injections were discontinued.

Boothby and Sandiford (1) and Thompson, Thompson, and Dickie (18) have found that the effects of thyroxin injections upon the basal metabolic rate can be observed for 30 to 60 days after treatment. The effects upon milk secretion are not so persistent. It would thus appear that the increase in metabolism is not the sole explanation for the increase in milk secretion since the trend of milk secretion does not follow the expected trend of metabolic rate. However, the body weights of the cows indicate that the metabolism is a factor since the animals remained constant in weight or increased very slightly during the periods of injection but gained more rapidly in the periods when they were not injected. This indicates that during the periods of injection the cows were using materials for milk secretion that otherwise would have been used for body tissue.

Turner (19) and Corner (2) have shown that the hormone galactin elaborated in the anterior lobe of the hypophysis has to do with controlling the secretion of the developed mammary gland. Evans and Simpson (3) and Van Horn (20) show that hyperthyroidism results in an increase in the activity of the hypophysis. It would thus appear that thyroxin stimulates milk secretion indirectly by causing an increased secretion of galactin from the anterior lobe of the hypophysis.

#### SUMMARY AND CONCLUSIONS

Two purebred Brown Swiss cows were injected intravenously with thyroxin alternately over three 28 day periods at a rate calculated to increase the basal metabolic rate by 10 per cent.

The quantity of milk secreted was weighed and analyses of the milk were made to determine the specific gravity, the weight of milk fat produced, and the percentages of milk fat, lactose, protein, solids-not-fat, lactalbumin, and lactoglobulin. Body weights were taken each 28 days.

Two purebred Holstein-Friesian cows were given intravenous injections

of thyroxin at seven day intervals for three weeks at a rate calculated to increase the basal metabolic rate by 30 per cent. Careful observations were made on the quantity of milk secreted by these cows.

An increase in milk secretion resulted from 25 mgm. thyroxin injections. They were most effective during the period of declining lactation just previous to the last few weeks of the lactation period. They were less effective at the peak of production and at the extreme latter end of the lactation period the effect was hardly significant.

The composition of the milk as shown by the constituents for which analyses were made was not significantly altered.

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# FACTORS AFFECTING ECONOMICAL MANUFACTURE, UNIFORMITY IN COMPOSITION AND QUALITY OF BUTTER

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The Bureau of Dairying, United States Department of Agriculture, co-operating with the Dairy Industry Division, University of California, started an extension program in 1922 for the standardization and improvement of butter in California. This program is based on the voluntary co-operation of creameries throughout the State who furnish samples of butter for scoring and other samples taken from the churn to be analyzed by the Kohman Method. The scoring and analyzing is done in the so-called "Butter Standardization Laboratory" where detailed records are kept and duplicate copies are returned to each creamery. The findings of this Laboratory are used as a guide in the standardization and improvement of the butter made in those creameries.

The first report on the progress of this program was published in Bulletin 443 of the California Agricultural Experiment Station by F. H. Abbott who continues to be in charge of the work he began twelve years ago. It is with his permission and assistance that this study is based upon the records of the Butter Standardization Laboratory.

The Laboratory analyzes, by the Kohman Method, from 4000 to 6000 samples of butter each year. These samples are furnished by creameries which manufacture about 60% to 70% of all the butter made in California so that the records reveal a fairly accurate picture of the quality of California butter.

Our present study is concerned with the factors influencing both the economical manufacture and the uniformity in both composition and quality of butter—quality as affected by composition. Such factors include the efficiency and conscientiousness of the employees, the efficiency of the machines used—but probably one of the most important factors is the overrun. The overrun reflects, in a large measure, all of the other factors which affect both economical manufacture and uniformity of product. The butter manufacturer readily learns that other factors being equal, an increase in the overrun reduces the cost of manufacture and increases his profits. Therefore, his problem becomes one of maintaining the maximum overrun. This maximum overrun is restricted by both State and Federal laws which require not less than 80.0% fat in butter. The result of these factors is an attempt by the manufacturer to maintain an overrun which puts on the market a butter containing as close to 80% fat as his facilities will permit.

The records of the Butter Standardization Laboratory show (Table 1) that the average fat content of California Butter is approximately 80.5%.

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This is considerably lower than the figures usually found in the literature. Five recognized references listed in table 1 give five different "averages" for the fat content of butter, namely, 81.5%, 82.41%, 81.12%, 81.31% and 83.0%. However, Hunziker<sup>1</sup> suggested the possible limitations of his figures by saying "American creameries that standardize the percentage composition of their butter, usually endeavor to secure an overrun that approaches as much as possible the maximum theoretical overrun attainable

TABLE 1  
*Average percentage composition of butter*

SOURCE	NUMBER OF SAMPLES	% FAT	% WATER	% SALT	% CURD
California (1931) (1)	5000	80.60	15.96	2.77	0.67
California (1932) (1)					
Gathered Cream	1500	80.49	16.13	2.70	0.68
Whole Milk	2500	80.53	16.32	2.50	0.65
Hunziker (2)		81.5	15.0	2.5	1.0
Hunziker (2)		80 to 81	15.3 to 15.9	2.5 to 3.5	1.0 to 1.25
Thompson, <i>et al.</i> (3)	695	82.41	13.90	2.51	1.18
Fundamentals of Dairy Science (4)	573	81.12	15.46	2.44	0.98
Eekles, Combs and Macy (5)	1000	81.31	15.35	2.31	1.03
Judkins and Smith (6)		83.0	13.9	3.0*	1.1

\* Recorded as ash in the reference and no mention made of salt.

(1) Taken from the records of the Butter Standardization Laboratory.

(2) Hunziker, O. F.: The Butter Industry, 2nd Edition, 1927, page 450, 451.

(3) Thompson, S. C., Shaw, R. S., and Norton, R. P.: The Normal Composition of American Creamery Butter. U. S. D. A., B. A. I., Bul. 149 (1912).

(4) Associates of Rogers: Fundamentals of Dairy Science, 1928, page 35.

(5) Eekles, C. H., Combs, W. B., and Macy, H.: Milk and Milk Products (1929), page 201.

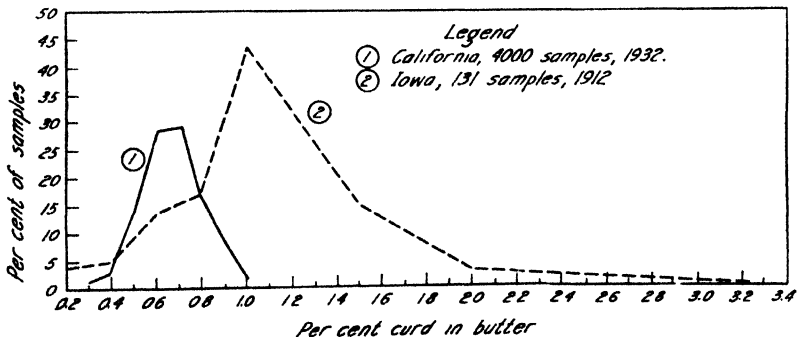
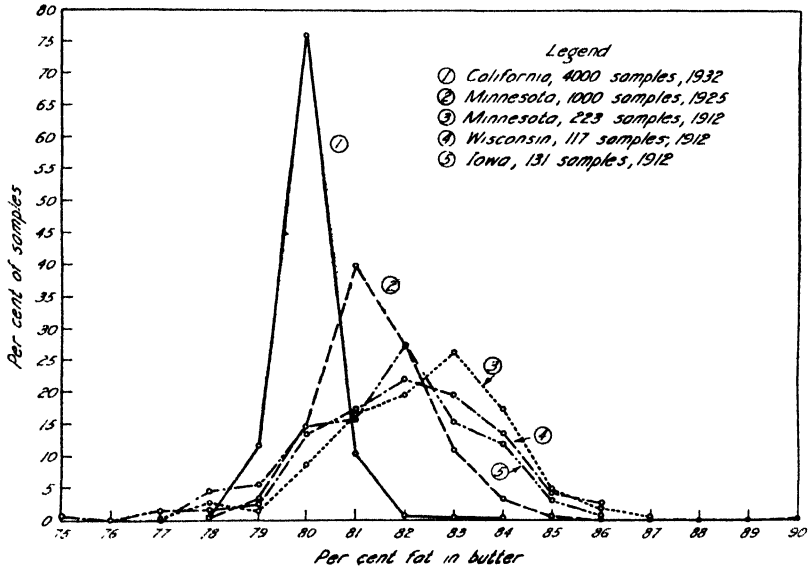
(6) Judkins, H. F., and Smith, R. W.: The Principles of Dairying (1931), page 27.

under the Federal requirement — —." Again in Fundamentals of Dairy Science, page 34, appears "The limits of variations are gradually becoming narrower due to the enforcement of Federal and State standards and to more nearly uniform methods taught by the State dairy school." These facts are demonstrated in figure 1 which shows the percentage distribution, according to fat content of samples analyzed in 1912, 1925, and 1932.

This relationship suggests a statistical treatment with narrower class intervals. The distribution of 4000 samples analyzed in the Butter Standardization Laboratory during 1932 appears in table 2 where the class-intervals give a smooth curve without minimizing the points of special interest, such as the percentage of samples which are only slightly below the State and Federal requirement of 80.0% fat.

<sup>1</sup> Hunziker, O. F.: The Butter Industry, 2nd Edition, 1927, page 451.

About 13% of all the samples appear in those groups containing less than 80% fat and less than 6% in groups containing more than 81.3% fat. Thus the narrow range of 80% to 81% fat is established for the majority of the butter. In other words, the normal percentage of fat in butter is in close agreement with the average percentage given in table 1. Furthermore, the average of the figures given in the literature and quoted in table 1 is calculated as 81.9% fat which falls in the groups including only 6% of the samples tabulated in table 2.



This lower fat content of 80.5% as compared with 81.9 means an increased overrun with a consequent increase in profit for the manufacturer. For example, a manufacturer who makes only 300,000 lbs. of butter per year

TABLE 2  
*Percentage distribution of 4,000 samples according to their fat content*

PER CENT FAT	78.00 TO 79.00	79.01 TO 79.74	79.75 TO 79.94	79.95 TO 80.54	80.55 TO 80.84	80.85 TO 81.04	81.05 TO 81.34	81.35 TO 84.00
	%	%	%	%	%	%	%	%
Whole milk	0.6	4.8	6.2	42.3	25.7	10.7	5.1	4.6
Gathered	0.4	8.2	5.5	40.1	19.4	12.1	8.5	5.8
All creameries	0.5	6.0	6.0	41.5	23.4	11.2	6.4	5.0

should have a manufacturing cost of 2.9¢ per lb. according to a survey of 115 Iowa creameries during 1921 and 1922. This 300,000 lbs. of butter having an average fat content of 81.9% would require 245,700 lbs. of fat which would cost \$53,562 at 21.8¢ per lb.—the average quotation of the San Francisco market for 1932. The cost of making this butter would be \$8,700 and the market value at 21.8¢ would be \$65,400. This leaves a profit of \$3,138 for the year of 1932. If this same manufacturer purchased the 245,700 lbs. of fat at the same cost of \$53,562, but controlled the fat content at 80.5% he would make 305,217 lbs. of butter having a market value at 21.8¢ per lb. of \$66,537. Assuming the same cost of manufacture, this would leave \$4,275 profit, an increase of 36% over the profit from butter containing the higher per cent fat.

While the fat content of the butter is the important factor in determining the overrun, the buttermaker at the churn usually controls it by a moisture test. This point of view does not give due credit to the salt content and curd content of the butter—both of them being as important in the control of overrun as in the moisture content. It will be seen from table 1 that the average salt content of butter is quite uniformly established at 2.5%.

TABLE 3  
*Percentage distribution of 4,000 samples according to their curd content*

RANGE OF PER CENT CURD	.20 TO .34	.35 TO .44	.45 TO .54	.55 TO .64	.65 TO .74	.75 TO .84	.85 TO .94	.95 TO 1.04
	%	%	%	%	%	%	%	%
Whole milk	1.3	2.6	14.4	29.4	28.3	15.1	7.0	1.9
Gathered	0.9	1.6	10.1	25.7	29.9	19.7	9.3	2.8
Total samples	1.1	2.2	12.8	28.1	28.9	16.8	7.9	2.2

However the curd content is commonly given as 1%—especially when speaking of what Hunziker calls the “curd by difference,” which includes all of the solids-not-fat in the butter. This is not in agreement with the figures for California butter as shown by table 1. Again table 3 shows the distribution of 4000 samples received during 1932 in class intervals of their

curd content. The majority of the samples contain between 0.55% and 0.84% "curd by difference," with the average "curd by difference" content at 0.67%. It is significant that none of the samples contained more than 1.0% "curd by difference" and only 10.1% of the samples contained more than 0.85% "curd by difference."

Therefore, from a study of these records, it appears not only desirable but also practical for the butter manufacturer to so control his overrun that the fat content of the butter will always fall between 80% and 81%. In order to do this, it is necessary to use not only the moisture test, but preferably the Kohman Method for analyzing each churning of butter.



# THE RELATION OF VITAMIN D TO CALCIUM AND PHOSPHORUS RETENTION IN CATTLE AS SHOWN BY BALANCE TRIALS

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The fact that a calf has a definite vitamin D requirement and suffers from a deficiency of this factor in its ration has been demonstrated by the published work of Rupel, Bohstedt and Hart (1), Bechdel, Landsburg and Hill (2), Huffman (3), and by unpublished work of Gullickson (4) in which experimental "rickets" developed when vitamin D was withheld and disappeared when this factor was added to the ration. Rupel, Bohstedt and Hart (1), and Bechdel, Landsburg and Hill (2) have reported an increased ash percentage in certain representative bones, and an improvement in the concentration of calcium and inorganic phosphorus in the blood plasma following vitamin D therapy of animals suffering from experimental "rickets," which gives indirect evidence of a beneficial effect of vitamin D on the calcium and phosphorus retention, but studies to directly measure this relationship have not been made.

## EXPERIMENTAL METHODS

Ten-day mineral balance trials were employed for directly measuring the calcium and phosphorus retention. The mineral retention of normal calves was obtained first to be used for comparative purposes. Young, growing calves were then placed on basal experimental rations relatively low in calcium and phosphorus content, and deficient in vitamin D. Prairie hay was used as roughage for one group, and beet pulp for the other. The concentrates included corn, corn gluten meal, oats, corn starch, and a little wheat bran. For some animals the basal rations were supplemented with calcium, as calcium carbonate, and/or phosphorus, as monobasic sodium phosphate, and/or vitamin D, as viosterol (250 D), or sunshine. Balance trials were run periodically to follow any changes and to note the effect of the various experimental schedules on the calcium and phosphorus retention of the calves. On all calves that developed a vitamin D deficiency as indicated by a rachitic syndrome and a subnormal concentration of calcium and inorganic phosphorus in the blood plasma,

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<sup>1</sup> The data presented are taken from the thesis by G. Carroll Wallis in partial fulfillment of the requirements for the degree of Ph.D., University of Minnesota, 1934. The problem was suggested by the late Dr. C. H. Eckles who acted as advisor during the first part of the work. Published with the approval of the Director as Paper 1308, Journal Series, Minnesota Agricultural Experiment Station.

particular attention was paid to obtain a balance trial while the calf was still eating well, but after the deficiency was well established. Vitamin D was then added to the ration, and after the lapse of a reasonable length of time, another trial was run.

Except when the experimental schedule required exposure to sunshine the animals were kept indoors in box stalls. A large indoor pen was available for additional exercise. Feeding was done individually and an accurate record kept of actual consumption. All feeds were analyzed for nutrient and calcium and phosphorus content before they were included in the ration. Weights and measurements of height at withers were taken periodically and the rations adjusted at 30-day intervals to furnish somewhat more than the nutrient requirements for growth given by the Minnesota Standard published by Eckles and Gullickson (5). The total calcium and inorganic phosphorus content of the blood plasma were determined from successive 3-day composite samples at least once every thirty days.

Standard methods were followed in conducting the balance trials. In order to accommodate steers in the metabolism stall special equipment was designed,<sup>2</sup> as shown in plate I. A heavy rubber funnel was made from a



PLATE I

E 201 in metabolism stall showing special equipment designed for collecting urine in balance trials.

<sup>2</sup> Credit is due Mr. M. E. Mattison, Curator of the Biochemistry Division, for assisting in designing and making this device.

large automobile inner tube. The top was clamped between two tape-covered metal rings which were then bolted together and bent somewhat to conform to the contour of the animal's body. The funnel opening was held in place over the opening of the animal's sheath by straps around the body. A small-bore, heavy-walled rubber tubing connected to a stopcock cemented into the lower end of the funnel, served to conduct the urine to the collecting pan. The calcium and phosphorus analyses of the excreta and feedstuffs were carried out by the methods of Morris, Nelson, and Palmer (6).

Vitamin D assays using rats were made on the two lots of prairie hay and the beet pulp used in the calf feeding work. The method followed was that given in U.S.P. X Interim revision for the assay of cod liver oil for vitamin D.

#### OBSERVATIONS

*The calcium and phosphorus retention of normal calves:* Three Holstein calves, E-180, E-183, and 436 and one Jersey calf, E-181 were used in this work. The ration included prairie hay and a concentrate mixture composed of the common grains and grain by-products. The calves had been exposed to sunshine during the summer and otherwise cared for in the customary manner. They appeared normal and showed a normal concentration of calcium and inorganic phosphorus in the blood plasma. During the balance trials the animals were exposed to sunshine on favorable days. As shown in table 1, these calves, seven to nine months of age, retained an average of 4.17 grams of calcium and 2.61 grams of phosphorus daily.

*Experiments using hay as roughage:* As soon as the balance trials under normal conditions had been completed, these calves and E-182, a grade Holstein, were started on their respective experimental schedules. All the animals were given an allowance of prairie hay which represented about the amount they would consume *ad libitum*. E-180, E-181, and E-182 ran parallel on a basal ration adjusted to furnish 30 grams of calcium and 15 grams of phosphorus daily per 1000 pounds of live weight. E-180 was kept indoors, E-181 was allowed sunshine exposure on favorable days, E-182 was kept indoors but given a vitamin D supplement of 2 cc. of viosterol (250 D) daily. E-183 and 436 were paired on a similar basal ration but with the phosphorus intake increased to 30 grams daily per 1000 pounds of live weight. E-183 was kept indoors while 436 received sunshine exposure.

After about three months on the experimental program, a series of balance trials were run to note any possible effect of the various procedures on the retention of calcium and phosphorus although none of the animals were showing manifestations of a vitamin D deficiency. The

TABLE 1  
*The results of mineral balance trials showing the calcium and phosphorus retention of four normal calves*

ANIMAL NO.	AGE AT BEGINNING	WEIGHT	TOTAL SUNSHINE	BLOOD PLASMA		MINERAL BALANCE (10 DAY PERIOD)					
				Total Ca	Inorganic P	Ca			P		
						Inake	Outgo	Balance	Inake	Outgo	Balance
	days	lbs.	hrs	mgm. per 100 cc.	mgm. per 100 cc.	gms.	gms.	gms.	gms.	gms.	gms.
E-180	290	479	6	9.91	7.46	108.4	53.8	+54.6	77.8	44.9	+32.9
E-181	288	397	6	10.41	7.32	95.6	64.4	+31.2	69.4	47.9	+21.5
E-183	220	415	2	10.48	7.43	119.1	59.4	+59.7	119.6	91.1	+28.5
436	269	465	2	10.15	7.17	132.5	110.9	+21.6	135.1	113.4	+21.7
Average (10 days)								- 41.7			+ 26.1
Daily average								+ 4.17			+ 2.61

TABLE 2  
*The results of mineral balance trials showing the calcium and phosphorus retention of calves receiving prairie hay as roughage, with and without mineral and vitamin D supplements*

ANIMAL	AGE AT BEGINNING	WEIGHT	SUPPLEMENTS		BLOOD PLASMA		MINERAL BALANCE (10-DAY PERIOD)					
			Mineral	Vitamin D	Total Ca	Inorganic P	Ca			P		
							Intake	Output	Balance	Intake	Output	Balance
	days	lbs.			mgm. per 100 cc.	mgm. per 100 cc.	gms.	gms.	gms.	gms.	gms.	gms.
E-180	431	670	None	None	10.94	5.85	210.3	133.3	+ 77.0	96.4	57.1	+ 39.3
E-182	394	677	None	Vioosterol <sup>1</sup>	10.68	7.09	210.7	140.0	+ 70.7	96.4	53.2	+ 43.2
E-183	318	580	Phosphorus	None	9.51	8.03	179.8	68.6	- 111.2	174.0	128.6	+ 45.4
436	364	622	Phosphorus	Sunshine <sup>2</sup>	10.46	7.72	190.3	98.9	+ 91.4	182.1	150.2	+ 31.9
E-169	610	871	None	Vioosterol	9.98	8.13	113.5	48.2	+ 65.3	113.3	78.0	+ 35.3
E-169	680	935	None	None <sup>3</sup>	9.61	7.30	132.7	57.9	+ 74.8	117.8	79.9	+ 37.9
E-170	592	738	Calcium	None	9.45	5.85	373.6	498.7	+ 74.9	91.0	66.3	+ 24.7
E-170	662	787	Calcium	Vioosterol <sup>4</sup>	10.62	4.98	664.7	626.8	+ 37.9	96.5	72.5	+ 24.0
Average (10 days)									+ 75.4			+ 35.2
Daily average									+ 7.54			+ 3.52

<sup>1</sup> Two cc. viosterol daily during entire experiment.

<sup>2</sup> Sunshine exposure during entire experiment.

<sup>3</sup> Vioosterol removed 17 days previous to this trial.

<sup>4</sup> Five cc. viosterol daily added 17 days previous to this trial.

results of these trials given in table 2 show essentially normal retentions of calcium and phosphorus for all the calves. The viosterol supplement of E-182 had not increased her mineral retention above that of E-180, neither had the sunshine exposure of 436 made possible larger retentions for this animal than for E-183. Likewise the increased phosphorus intake of E-183 and 436 had not brought about an increased retention of this element as compared with that of E-180 and E-182. As all of these animals continued through the winter in essentially normal condition no further balance trials were conducted on this group.

The results obtained with E-169 and E-170, grade Holstein females, are also given in table 2. These animals were available from another experiment in which they had developed a rachitic syndrome on a low-calcium-low-phosphorus ration under indoor conditions. E-169 had been growing vigorously throughout the summer after receiving a viosterol supplement with no further change in her experimental schedule. E-170 had shown slow but persistent improvement after the addition of  $\text{CaCO}_3$  to her ration. Both animals were receiving prairie hay. The results from the first balance trial with each animal indicate that the viosterol was enabling E-169 to retain adequate amounts of calcium and phosphorus from her limited intake, and that E-170 was retaining adequate amounts by virtue of the increased calcium intake without additional vitamin D. The viosterol was now removed from the ration of E-169 and added to that of E-170 at the rate of 5 cc. daily. After seventeen days, balance trials were again run. E-169 was still retaining approximately the same amount of calcium and phosphorus. The added viosterol had not improved the mineral retention of E-170, in fact, it was somewhat less but still within the limits of variations reported for normal animals.

The results of the balance trials run on this group of calves receiving hay as roughage are essentially normal in all cases. None of the calves showed the characteristic symptoms of a vitamin D deficiency. As E-180 and E-183 were on the basal schedule without additional vitamin D for six to seven months it would seem that a reasonable length of time had elapsed in which to deplete the possible vitamin D stores of a young calf at weaning time, hence it seemed more likely that all the animals were getting this factor from some other source. In view of the fact that several investigators have found a varying, yet appreciable, amount of vitamin D in different kinds of hay, it seemed that the prairie hay was the most likely source of an appreciable amount of this factor in the experimental schedule of the control calves on the basal ration. To check this point with approved methods, the prairie hays and beet pulp used as roughage were subjected to vitamin D assays by use of laboratory animals. Both lots of prairie hay were found to carry appreciable amounts of vitamin D which affords a logical explanation for the essentially normal

condition and normal mineral retentions of the calves on the basal ration and the lack of any appreciable differences in the retentions of those getting varying amounts of vitamin D supplements from different sources.

The vitamin D assays with laboratory animals were designed to be qualitative rather than quantitative. Preliminary tests indicated that rats would readily consume the test diets containing 45 per cent of powdered prairie hay and 25 per cent of beet pulp which corresponded to the levels of these materials in the calf rations. The calcium/phosphorus ratio of the rachitogenic diet was changed but slightly when the test materials were added, the tendency being for a wider ratio.

The results of the line test assays carried out according to the requirements of the U.S.P. X Interim revision of the assay of cod liver oil for vitamin D are shown in table 3.

TABLE 3

*The results of vitamin D assays by laboratory animals, showing the food intake and the degree of healing for the various test materials*

TEST MATERIAL	AMOUNT IN DIET	NUMBER OF RATS	AVERAGE DAILY FOOD INTAKE			AVERAGE DEGREE OF HEALING
			Rachito- genic period	Assay period		
				Test diet	Latent Rachito- genic diet	
	<i>per cent</i>		<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	
Prairie Hay—Lot 45	45	12	6.6	9.8	8.4	1.46
Prairie Hay—Lot 45	30	4	5.9	8.3	7.1	0.75
Prairie Hay—Lot 45	15	4	5.9	7.8	7.2	0.00
Prairie Hay—Lot 55	45	13	6.0	8.5	8.5	2.38
Beet Pulp—Lot 7	25	5	5.3	6.0	6.7	0.00
Rickets Diet—21 day controls		2	5.9			Wide Metaphyses
Rickets Diet—31 day controls		2	6.3		6.5	Wide Metaphyses

<sup>1</sup> The line tests were assigned values of 0, +, ++, etc., and the numerical value of pluses averaged to give the data in this column.

*Experiments using beet pulp as roughage:* The animals used in this experiment were E-201, a grade Shorthorn steer about one year old, received from a farm in a vitamin D deficient condition; E-185, a grade Guernsey female thirteen months old; and E-194, a purebred Holstein, eleven months old.

After being received from the farm, E-201 was maintained for about six weeks under indoor conditions on a diet similar to that received on the farm. This included two pounds of prairie hay daily and a grain mixture composed of corn, oats, and corn gluten meal. The calf was then in excel-

lent experimental condition for determining the calcium and phosphorus retention of a growing animal suffering from a vitamin D deficiency. The deficiency was evidenced by his rachitic-like condition and the low concentration of calcium and inorganic phosphorus in the blood plasma as noted in table 4, the first balance trial for E-201. After this trial the ration remained the same except for the addition of 5 cc. of viosterol (250 D) daily. The response to this treatment was prompt and marked. The blood picture was normal within three weeks, the stiffness had largely disappeared, the animal was more alert and active, and the appetite was improving. At the end of four weeks he was placed on a balance trial again to ascertain the nature of the calcium and phosphorus retention on the same mineral level but with the animal rapidly approaching a normal condition following the vitamin D therapy. The improvement in calcium retention from 1.44 grams to 6.82 grams daily was nearly five-fold, while the increase in phosphorus retention from 1.10 grams to 2.68 grams daily was slightly less than three fold.

Shortly after the completion of this second trial observations indicated that the animal was apparently normal, whereupon the viosterol was removed from the ration, the experimental schedule otherwise being continued as before. There was a steady decline in the blood plasma calcium from 10.15 to 6.12 mgm., per 100 cc. and in inorganic phosphorus from 8.26 to 5.05 mgm., during the next three and one-half months, at which time the other rachitic-like symptoms began to appear. These observations indicated that body stores of vitamin D, and/or calcium and phosphorus had been depleted and the animal was again in a vitamin D deficient condition. At this time a third balance trial was run, followed by a fourth about five weeks after the ration had been again supplemented with 5 cc. of viosterol. As noted in table 4, the increase in calcium and phosphorus retention was even more striking than before and serves to indicate the important rôle played by vitamin D in promoting the mineral retention of vitamin D deficient calves.

It is conceivable that the level of mineral intake may influence the effect of vitamin D on mineral retention. E-185 and E-194 developed a severe rachitic-like condition on a ration of beet pulp and grain mixture furnishing a very limited calcium intake. A slight temporary improvement in the blood plasma calcium resulted when a  $\text{CaCO}_3$  supplement was added to bring the intake of E-185 to 40 grams daily and E-194 to 50 grams daily. By the end of a month it was declining again and the physical condition was very poor. E-185 was showing considerable anorexia, and E-194 was also slightly off feed so that the mineral intake indicated in the balance trials run at this time is somewhat lower than the prescribed amounts previously consumed. The results are shown in table 4 as the first balance trial for each animal, respectively. The inability of vitamin D deficient

TABLE 4  
*The results of mineral balance trials showing the calcium and phosphorus retention of calves, receiving beet pulp as roughage, with and without mineral and vitamin D supplements*

ANIMAL	AGE AT BEGINNING	WEIGHT	SUPPLEMENTS		BLOOD PLASMA		MINERAL BALANCE (10 DAY PERIOD)					
					Total Ca	Inorganic P	Ca			P		
			Mineral	Vitamin D			Intake	Output	Balance	Intake	Output	Balance
	days	lbs.			mgm. per 100 cc.	mgm. per 100 cc.	gms.	gms.	gms.	gms.	gms.	gms.
E-201		527	None	None	6.42	3.56	71.7	57.2	+ 14.5	83.8	72.8	+ 11.0
E-201		539	None	Vioosterol <sup>1</sup>	10.09	6.87	96.4	29.2	+ 68.2	83.8	57.0	+ 26.8
E-201		715	None	None <sup>2</sup>	6.12	5.05	85.8	79.7	+ 6.1	122.2	116.5	+ 5.7
E-201		786	None	Vioosterol <sup>3</sup>	9.29	7.94	102.9	50.1	+ 52.8	119.8	96.4	+ 23.4
E-185	475	485	CaCO <sub>3</sub>	None	8.41	3.13	271.2	292.0	- 20.8	38.5	48.8	- 10.3
E-185	538	461	CaCO <sub>3</sub>	Vioosterol <sup>4</sup>	11.15	4.98	240.4	184.0	+ 56.4	38.9	21.5	+ 17.4
E-194	430	575	CaCO <sub>3</sub>	None	8.30	3.40	356.2	335.5	+ 20.7	48.8	45.6	+ 3.2
E-194	462	625	CaCO <sub>3</sub>	Vioosterol <sup>5</sup>	11.11	6.41	456.0	333.0	+ 122.4	72.1	32.3	+ 39.8

<sup>1</sup> Started feeding 5 cc. of viosterol daily 33 days previous to this trial.

<sup>2</sup> Viosterol feeding discontinued approximately 3½ months previous to this trial.

<sup>3</sup> Started feeding 5 cc. of viosterol daily 40 days previous to this trial.

<sup>4</sup> Started feeding 5 cc. of viosterol daily 53 days previous to this trial.

<sup>5</sup> Started feeding 5 cc. of viosterol daily 23 days previous to this trial.

calves to make normal mineral retention on rations supplying an abundance of calcium and normal phosphorus is indicated by the fact that E-185 was in negative calcium and phosphorus balance while E-194 was slightly better than in equilibrium.

A second balance trial was run on each animal after the ration had been supplemented for some time with viosterol. Under the influence of the added vitamin D both animals now showed very satisfactory positive balances of both calcium and phosphorus, as may be noted in the second trial for each animal as recorded in table 4.

Although the appetites were now good, the nutrient and mineral intake of E-185 was held down to the same amount that she consumed on the previous trial, but for unavoidable reasons the mineral intake, especially the calcium, was somewhat larger for E-194 in the second trial than in the first. However, the calcium intake of E-194 was liberal, and more than sufficient in both trials so that the variation undoubtedly does not introduce any appreciable difficulty in interpreting the results. The increase in the calcium intake was approximately 100 grams for the 10-day period, while the increase in retention was about 102 grams. The tendency is for the percentage of mineral retention to decrease with an increase in the intake, but even by assuming that the retention of the extra 100 grams of calcium in the second trial would have been at the same rate as in the first trial there would have been an increase of only about 5.81 grams in the second trial without the added vitamin D. The fact that over 100 grams of extra calcium were retained in the second trial indicates clearly the beneficial effect of the added vitamin D.

The results secured with E-185 and E-194 substantiated the observations already made on E-201 and indicate that a liberal calcium intake will not suffice to promote adequate mineral retention in calves suffering from a vitamin D deficiency.

*The relation of vitamin D to aphosphorosis:* Two grade Holstein females, E-187 and E-190, twelve and ten months of age, respectively, were used in an attempt to study the effect of vitamin D on the mineral retention of calves suffering from aphosphorosis. The basal ration furnished liberal calcium but a restricted amount of phosphorous, and was typical of those upon which aphosphorosis develops except that vitamin D-free beet pulp was substituted for the usual hay roughage and the calves were kept indoors. By the end of two months it began to appear that uncomplicated aphosphorosis could be obtained only in the presence of at least some vitamin D. The blood plasma calcium as well as the inorganic phosphorus began to decline and the physical condition more nearly resembled that of the rachitic syndrome than simple aphosphorosis. There was no evidence of pica. The onset of physical disturbances was rather sudden and severe with E-190 so that she had to be dropped from the experiment. A balance

TABLE 5  
*The results of mineral balance trials on E 157 showing the calcium and phosphorus retention on the basal ration,<sup>1</sup> basal ration plus phosphorus, and basal ration plus viosterol.*

TYPE OF RATION	AGE AT BEGINNING	WEIGHT OF ANIMAL	BLOOD PLASMA		MINERAL BALANCE				(10 DAY PERIOD)			
			Total Ca	Inorganic P	Ca				P			
					Intake	Outgo	Balance		Intake	Outgo	Balance	
	days	lbs.	mgm. per 100 cc.	mgm. per 1.00 cc.	gms.	gms.	gms.		gms.	gms.	gms.	
Basal	478	694	8.66	3.45	294.5	237.2	- 57.3		77.1	53.5	+ 23.6	
Basal plus P <sup>2</sup>	501	700	6.12	5.26	- 103.9	127.6	- 23.7		41.1	63.2	- 22.1	
Basal plus viosterol <sup>3</sup>	574	630	10.73	7.73	270.5	192.2	+ 78.3		80.7	34.6	+ 46.1	

<sup>1</sup> Basal ration furnished liberal Ca, restricted P, typical of those producing aphosphorosis except that beet pulp was substituted for the customary hay roughage, and animal was kept indoors. Alterations resulted in development of rachitic syndrome rather than uncomplicated aphosphorosis.

<sup>2</sup> NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O added to increase P intake to 18 grams daily 7 days previous to this trial. Animal badly off feed during this trial.

<sup>3</sup> Animal received viosterol for 2 months previous to this trial.

trial was run on E-187 although her condition was not that of simple aphosphorosis. The results of this trial on the basal ration are shown in table 5. The mineral retention is about what might be expected for a mildly rachitic animal.

A second trial was run about a week after a supplement of monobasic sodium phosphate had been added to her ration, to study the effect of added phosphorus on the calcium and phosphorus retention of vitamin D deficient animals, and to learn the nature of the mineral retention of such animals receiving a liberal intake of both calcium and phosphorus. The animal became worse rapidly and went off feed so badly that a satisfactory balance trial to indicate the effect of added phosphorus was not obtained. The results of the trial are shown in table 5. The fact that a severe breakdown seemed to be hastened would indicate that the effect undoubtedly was not very favorable.

The phosphorus supplement was now removed from her ration and viosterol added. As the animal was nearing recovery a third balance trial was run with the ration adjusted to duplicate that of the first trial except for the added viosterol. The results given in table 5 show a considerable improvement in both the calcium and phosphorus retention over that obtained when the animal was mildly deficient in vitamin D and thus substantiate the previous observations as to the favorable effect of vitamin D.

#### DISCUSSION

The favorable effect of vitamin D on the calcium and phosphorus retention of calves suffering from a vitamin D deficiency is strikingly shown by the results obtained with E-201, E-185, and E-194. The vitamin D deficiency of these animals was indicated by subnormal concentrations of calcium and inorganic phosphorus in the blood plasma, stiffness, bending of the knees, swelling of the knee, hock and pastern joints, humping of the back, and often some inanition. As shown in table 6, the average daily

TABLE 6  
*The calcium and phosphorus retention of calves before and after vitamin D administration*

ANIMAL NUMBER	MINERAL BALANCE (TEN-DAY PERIOD)			
	Before feeding viosterol		After feeding viosterol	
	Ca	P	Ca	P
	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>
E-201 . . . . .	+ 14.4	+ 11.0	+ 68.2	+ 26.8
E-201 . . . . .	+ 6.1	+ 5.7	+ 52.8	+ 23.4
E-185 . . . . .	- 20.8	- 10.3	+ 56.4	+ 17.4
E-194 . . . . .	+ 20.7	+ 3.2	+ 122.4	+ 39.8
Average (10-day period) . . . . .	+ 5.1	+ 2.4	+ 74.9	+ 26.8
Daily average . . . . .	+ 0.51	+ 0.24	+ 7.49	+ 2.68

retention of these calves at an age when bone mineralization should have been progressing rapidly was only 0.51 grams of calcium and 0.24 grams of phosphorus. When viosterol was added to supply vitamin D, and without any other appreciable change in the ration, there was a marked increase in the calcium and phosphorus retention in every case, the daily average now being 7.49 grams of calcium and 2.68 grams of phosphorus. These amounts represent essentially normal retentions. The increase brought about by the vitamin D administration in from three to seven weeks was approximately fourteen-fold for calcium and eleven-fold for phosphorus. Coincident with the marked improvement in the mineral retention there was a prompt return of the calcium and inorganic phosphorus of the blood plasma to normal concentrations, and a corresponding improvement in the physical well-being of the calf. With such decided and consistent results there can be no doubt of the important rôle played by vitamin D in promoting the retention and utilization of calcium and phosphorus by calves under these conditions.

Increasing the mineral content of the ration of vitamin D deficient calves had no such favorable influence on the mineral retention. Increasing the calcium intake of E-185 and E-194 to 40-50 grams daily failed to promote adequate mineral retention, in fact, E-185 was in negative balance. Although the evidence for the effect of added phosphorus in the case of E-187 is not as conclusive, the fact that adding a liberal amount of phosphorus to the ration of this animal suffering from a mild vitamin D deficiency did not prevent, and may even have hastened, a severe breakdown so that a satisfactory balance trial could not be obtained would cast considerable doubt on the possibility of any beneficial effects of the added phosphorus.

Further information on the mineral retention of normal growing calves is also afforded. Data for the mineral retention of four normal calves, seven to ten months of age, have been given in table 1. As the calves in the experimental group receiving prairie hay as roughage completed the experiment in essentially normal condition, and the rations were similar to those commonly fed in practice, it would seem that these results might also be considered as representative of essentially normal retentions. The data for this group have been given in table 2. When the results of the above two groups are averaged together, the grand average of all the balances with essentially normal calves shows an average daily retention of 6.42 grams of calcium and 3.22 grams of phosphorus. These results corroborate the work of Lindsey, Archibald, and Nelson (7), who obtained normal retentions of 7.74 grams of calcium and 3.52 grams of phosphorus daily for a high calcium group and 4.45 grams of calcium and 2.15 grams of phosphorus daily for a low calcium group.

The average normal daily retention of 6.42 grams of calcium and 3.22

grams of phosphorus obtained in this work is almost exactly in a two to one ratio irrespective of the varying amounts in the ration fed. This closely approximates the ratio of these two elements in the bodies of growing and mature cattle. Lindsay, Archibald, and Nelson (7) noted the same relationship in their work with normal animals. It is of interest to note that in this work the slight average daily positive balances of 0.51 grams of calcium and 0.24 grams of phosphorus shown by the rachitic calves are also in an approximately two to one ratio. Also, that the loss of calcium and phosphorus in the case of E-185 showed this ratio. Such observations indicate an ultimate interdependence between the two elements and suggest that a deficiency in one might be responsible for a lack of retention of the other.

The fact that the calves receiving prairie hay as roughage continued throughout the experiment in an apparently normal condition irrespective of whether or not they received any vitamin D supplement, whereas those on a similar or even higher mineral intake but receiving beet pulp soon showed symptoms of a vitamin D deficiency, indicates that the prairie hay undoubtedly carried an adequate amount of the antirachitic factor, while the beet pulp contributed very little, if any. The vitamin D assays with laboratory animals furnished corroborative and conclusive evidence on the above points. When the two lots of prairie hay were fed to rachitic rats definite healing was obtained, although it was more pronounced for one lot than for the other. No signs of healing were evidenced when the beet pulp was fed.

The assays furnish a basis for estimating the amount of vitamin D supplied the calves by the hay contained in their rations. Rough calculations indicate that the average hay allowance supplied the calves with approximately 135 Steenbock units of vitamin D daily, which served to protect the calves from developing the rachitic-like syndrome on the level of mineral intake furnished by these rations. Just how much less may have sufficed to protect the animals can not be determined from these observations. These estimations indicate that the vitamin D requirement of this species is relatively small as compared with the requirement of infants as recently reported by Hess and Lewis (8), while the fact that an increased mineral intake and changes in the calcium/phosphorus ratio failed to protect the calves or initiate appreciable healing suggests the possibility that calves are less independent of vitamin D than the rat.

Some suggestions as to the amount and length of storage of vitamin D by calves are also indicated by the results obtained. The viosterol fed to the calves as a therapeutic agent supplied roughly 15,000 Steenbock units daily, which is over 100 times the amount furnished by the hay. The exact amount which would have proved sufficient is not known but if the vitamin D in the viosterol had been used efficiently and the excess stored, the animals should have accumulated sufficient for protection for a comparatively long

period of time. When the viosterol was removed from the ration of E-201 after the second balance trial the previous stores of vitamin D made during two and one-half months of viosterol feeding were depleted in about three and one-half months, as evidenced by his physical condition and the low concentration of calcium and inorganic phosphorus in the blood plasma. The time interval would undoubtedly have been shortened had the two pounds of prairie hay allowed part of the time been replaced with beet pulp all of the time. These observations suggest several possibilities. For instance, the vitamin D in irradiated ergosterol may not be the most effective for this species so that the excess available for storage was small, or it may be inefficiently absorbed, or if absorbed, it may be reexcreted. It is also possible that the calf has only limited ability for storing vitamin D at best. The observations on these points are very meager and the possibilities mentioned are made merely as suggestions. The results do indicate, however, that a young growing calf may make a storage of vitamin D under favorable circumstances which may be used as a protection against a deficiency of this factor for a varying length of time under adverse conditions.

#### CONCLUSIONS

The following conclusions may be drawn from the results of this investigation.

1. The calcium and phosphorus retention of vitamin D deficient calves is very markedly improved by the administration of vitamin D. The average calcium retention may be increased fourteen-fold and the phosphorus retention eleven-fold by vitamin D therapy.

2. Increasing the mineral content of the ration of vitamin D deficient calves has no favorable influence on the mineral retention.

3. Based on the limited evidence obtained, the average daily retention of normal calves approximated 6.50 grams of calcium and 3.25 grams of phosphorus.

4. Judging from the results of this experiment, calcium and phosphorus are retained by normal calves in approximately a two to one ratio regardless of variations in the mineral content of the ration. The statement is equally true for the small average daily retentions made by calves suffering from a vitamin D deficiency. These observations indicate an ultimate interrelationship between these two elements and suggest that a shortage of either one in the ration might act as a limiting factor in the retention of the other.

5. Prairie hay may carry appreciable amounts of vitamin D. Beet pulp, on the other hand, probably possesses very little, if any, antirachitic potency.

6. A young growing calf may store vitamin D under favorable conditions to be used as a protection against a deficiency of this factor for a varying length of time under adverse conditions.

7. Uncomplicated aphosphorosis does not develop when all sources of appreciable amounts of vitamin D are eliminated from rations otherwise similar to those which ordinarily bring about this condition.

8. Vitamin D acts to improve the mineral retention of calves suffering from a rachitic-like syndrome within at least three to seven weeks after its administration.

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## EFFECT OF HEATING MILK ON THE TIME WHICH THE CURDS REMAIN IN THE ABOMASUM OF CALVES\*

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Soon after coagulation of milk in the stomach the whey begins to separate from the curd. Syneresis of the coagulum, assisted by the peristaltic movements of the stomach, caused the curd to become firmer in texture for some time after the clot has formed. The abomasum of the sucking calf is rarely if ever empty of these curds. As the calf matures, however, and is forced to drink milk from a pail sufficient dilution of the milk sometimes occurs to prevent coagulation. In every case where the milk passes into the rumen on its way through the stomach the strength of the clot is diminished by dilution with the ingesta present there.

The normal healthy calf is physiologically adapted to take care of the curd formed regardless of its tenacity. Gastric juice continues to be secreted for a sufficient length of time to dissolve the nutrients from the coagulum needed for maintenance and growth. When digestion is nearly completed the flow of the digestive juices is retarded sufficiently to avoid wastage. But under abnormal conditions such as disease, the body functions are impaired. One of the first symptoms of a depressed condition is a diminution in gastric secretion. Necessarily, the food should be more digestible at such times. Earlier work (3), (5) has shown that soft curd milk is more digestible than hard curd milk. Brenneman (1), Wallen-Lawrence and Koch (6) have found that heating milk makes it more digestible.

These two viewpoints left the question unsettled as to just what relation heating bore to curd tension. If heating did reduce curd tension it was desirable to find out whether this artificial method of changing the toughness of the curd affected its rate of passage through the stomach in the same way as natural soft curd milk. Besides making these general applications we especially wanted to know the reaction of the calf's digestive system to heated milk.

### EXPERIMENTAL

Calves with gastric fistulas were used for these trials. The day before each trial was started the rumen of the calf to be used was emptied manually. Muzzles were then put on the calves and all feed withheld for 12 to

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18 hours preceding the test. At least one hour before the trial the rumen was washed several times with warm water until the washings were clear. The abomasum was palpated with the fingers to make certain that it was also empty. A stomach tube one-quarter inch in diameter was then inserted into the abomasum by way of the gastric fistula through the reticulo-omasal orifice. The external end of the tube was threaded through the plug used to close the fistula and the plug replaced in the fistula opening. This was done to minimize moisture and heat loss from the rumen.

Two liters of warm milk were then fed through the stomach tube which remained in place throughout the entire trial. Periodically, after feeding, milk was withdrawn from the stomach through this tube into a glass syringe for observation in order to determine the coagulation time of the milk. As soon as the milk had started to whey off, a sample of the gastric juice-whey mixture was drawn for analysis. Subsequent samples of juice were drawn at hourly intervals from the time of feeding.

These samples were tested for hydrogen-ion concentration, free acidity, and total acidity. An attempt was also made to test the peptic activity of the juice, but though two methods were tried each of which gave good results with artificial gastric juice, the results were unsatisfactory with the juice from calves. The milk may have modified the gastric juice so as to interfere with the tests. Probably the pepsin of the gastric juice was adsorbed to the casein.

Curd tensions on all milk fed were obtained before and after heat treatment by a modification of the Hill method (5). The principal difference in the curd test used and that described by Hill was that the authors used commercial rennin instead of pepsin and calcium chloride as the coagulant and that a series of tests were made at two-minute intervals after coagulation.

Milk treated in the following ways was tested for curd tension.

1. Untreated.
2. Boiled for 3 minutes on an oil bath.
3. Heated in an autoclave at 242° F. for 15 minutes.
4. Pasteurized in a vat pasteurizer at 142° F. for 30 minutes.
5. Condensed (plain). This milk was heated to 180° F. during the condensing process.

#### RESULTS

Typical results of heat treatment on milk are shown in table 1. It will be noted that boiling for three minutes on an oil bath lowered the curd tension in sample 1 to about one-fourth that of the original raw state. In sample 2 there is an even greater difference between the curd tensions of raw and boiled milk. In this case the curd of the boiled milk was very flocculent and when poured from the container broke easily into small

TABLE 1  
*Typical results of the effect of heat on the curd tension of skim milk*

COAGULATION TIME** (MINUTES)	CURD TENSION IN GRAMS*							
	SAMPLE 1			SAMPLE 2		SAMPLE 3		SAMPLE 4
	Raw	Boiled	Auto claved 242° F	Raw	Boiled	Raw	Pas- teur- ized	Plain Con- densed 180° F (re-diluted)
10	70	19	0	54	3	72	46	8
13	74	22	0	58	6	81	49	16
16		20	0	48	4	85	65	13
19	76	22	0	60	6	92	62	9
22	80	16	0	64	0		70	15
25	83	18	0	67	2	98	78	12
28	65		0	49		93	70	17
31			0					13

\* Sample 1 was mixed milk from six cows in the College dairy herd. This milk was used in the digestion experiments. Samples 2, 3 and 4 were obtained from different sources.

\*\* Elapsed time between the introduction of the coagulant and the curd tension reading.

pieces. Heating to 242° F. in an autoclave for 15 minutes lowered the curd tension to a point where it could not be measured with curd knives. Pasteurizing in a vat pasteurizer for 30 minutes at a temperature of 142° F. reduced the tension of the curd about 20 per cent.

Plain condensed skim milk which had been heated during the condensing process to 180° F. was tested for curd tension after being rediluted to the specific gravity of raw milk. Heating at this temperature had not reduced the curd to as friable a texture as that of the milk which had been autoclaved at 242° F. The curd tension of the rediluted plain condensed skim milk was less than that of boiled milk in sample 1 and more than that of boiled milk in sample 2. Pasteurizing milk at 142° F. for one-half hour markedly reduced the curd tension of hard curd milk.

According to Hill (5) pre-heating to 114° F. affected the curd tension only slightly. Heating to 197° F. reduced the curd tension to less than one-third of the original curd tension of the milk. When diluted, sterilized evaporated milk had a curd tension only about one-tenth that of the original milk. From Hill's studies it is plainly evident that heat reduced the curd tension, especially when the temperature reaches 180° F. or above.

#### EFFECT OF HEAT TREATMENT UPON THE TOTAL TIME MILK REMAINS IN THE ABOMASUM OF CALVES DURING DIGESTION

A total of thirty-two samples (two liters each) of raw, pasteurized, boiled and autoclaved milk were used in these trials. The results are shown in figure 1. At first the evacuation time was determined by palpation of

the stomach contents by passing the hand through the fistula opening and then further inserting two fingers into the abomasum. Later with experience it became possible to estimate the length of the digestion period with a fair degree of accuracy by palpating the abomasum through the medial wall of the rumen. However, the curd which remained after the trial was considered complete was removed and its dry weight determined.

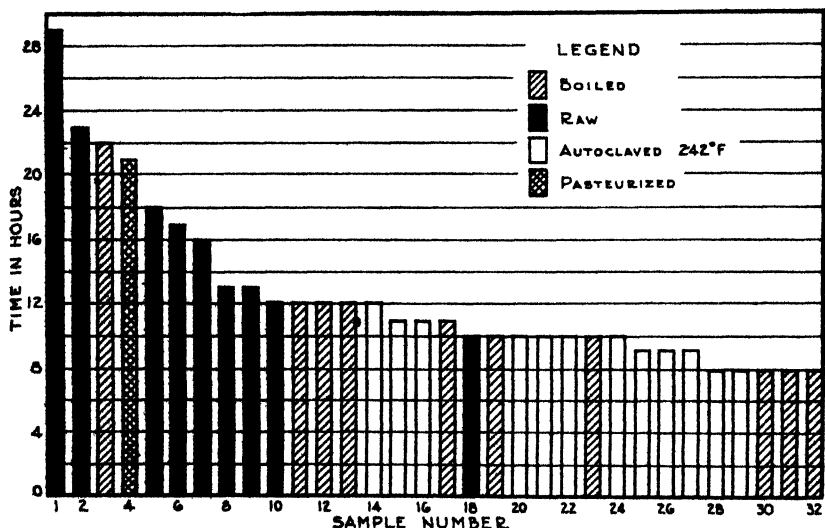


FIG. 1. EVACUATION TIME OF STOMACH WHEN CALVES WERE FED TWO LITERS OF SKIM-MILK. END OF DIGESTION DETERMINED BY PALPATION THROUGH FISTULA.

It is evident from figure 1 that a longer time is required for the liquefaction and removal (through the pylorus) of raw milk than boiled or autoclaved milk. Only one trial on pasteurized milk was carried to completion. This test very closely resembled those on raw milk. Boiling and autoclaving milk seem to affect its rate of evacuation from the stomach in much the same way. Both of these methods of heat treatment shortened the evacuation time from that required by either raw or pasteurized milk. The results of the 12 trials carried out with autoclaved milk are much alike, the emptying time of the stomach varying in length from eight hours to twelve hours. The trials with boiled and raw milk were rather uniform except in one case with boiled and two with raw milk which show the influence of the health of the calf on gastric response. These calves were apparently normal as judged by external appearances at the time of feeding. Most digestive disturbances, however, were followed by bowel disturbances, a few hours later.

The emptying time of the stomach usually varied with boiled milk from eight to twelve hours while raw milk required between twelve and eighteen hours. This was longer than was required in another series (4) in which only one liter of milk was used.

In the course of these studies with fistula-calves it was discovered that the curd could be removed from the abomasum without "upsetting" the calf. When the curd was tough, large masses could frequently be withdrawn through the reticulo-omasal orifice without being torn apart. At times curd weighing as much as 75 grams was removed in one piece in this manner. Efforts were then made to trace the digestion of raw and boiled milk by removing the remainder of the test meal after varying periods of time. The weights of the dry curd are listed in table 2.

TABLE 2

*Dry weights of curds removed from the abomasum of two calves at varying intervals after feeding two liters of skim milk*

WEIGHTS OF CURD IN GRAMS*						
RAW MILK				BOILED MILK		
3rd Hour	6th Hour	9th Hour	16th Hour	3rd Hour	6th Hour	
22.3	23.8	10.9	9.7	36.7	23.4**	
20.2	30.1	10.9		38.4**	25.3	
36.6		15.8		40.9	26.0	
38.0		24.9		43.9**	27.8**	
51.3**				53.9**		
61.9**						
65.1**						
Average	43.6	26.9	15.6	9.7	42.6	25.6

\* Dried in oven at 75 to 85° C. until weight was constant.

\*\* These samples were siphoned out after crumbling the curd with the fingers and pouring warm water into the abomasum. Siphoned samples are heavier as mucous and residual gastric juice are included.

Although yielding information as to the amount of curd in the stomach at different intervals of time the figures should not be taken as strictly comparable. The trials are too few in number and the curd was not always drawn in the same manner. Because the toughness of the curd increases as the digestion period progresses, it was easier to withdraw a sample which had been in the stomach nine hours than one which had been digesting three hours or even six hours. With proper care six hour samples could usually be removed in one or two pieces, and occasionally curd which had been digesting for only three hours could be removed almost intact. The curd from boiled milk crumbled in pieces if one attempted to withdraw

it after three hours. Occasionally the boiled milk curd broke up when handled after digesting six hours. The samples that crumbled too much were further triturated with the fingers until they were small enough to be siphoned out after pouring warm water into the abomasum. Nine hour weights on boiled milk are not included. Most samples had usually left the stomach at this time.

The limited data indicate that both raw and boiled milk "digest" at much the same rate during the first three to six hours. After this the boiled milk "digests" at a much faster rate. The optimum pH for peptic activity is not reached until about the fifth hour. Perhaps boiled milk is liquefied faster, once the pH of the stomach becomes optimum, because the curd is loose and more friable and permits easier penetration of the digestive fluid.

The method of experimentation used is open to two criticisms: (1) the milk was not mixed with saliva as occurs in sucking or drinking and (2) the fistula may have modified the normal process of digestion. Neither criticism seems especially important to the authors in that the same technique was used in all trials. Besides, according to Dukes (2), the enzymatic action of the saliva of ruminants is relatively unimportant in predigesting proteins, fats and sugars. As to the effect of the fistula on digestion, the

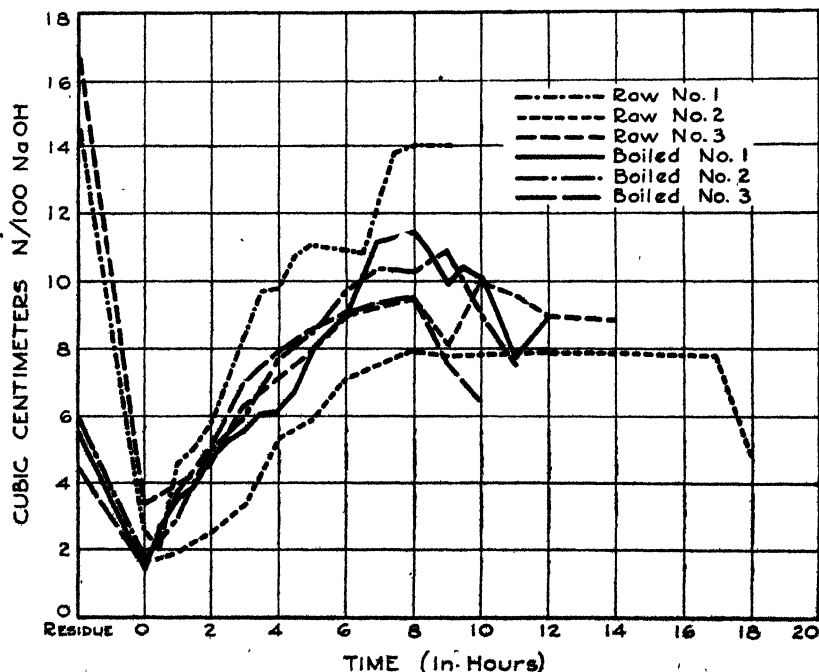


FIG. 2. EFFECT OF BOILING MILK UPON THE TOTAL ACIDITY OF THE GASTRIC JUICE.

acidity curves would indicate that normal physiological processes were occurring in the stomach. Any effect from moisture loss was reduced by replacement of the wooden plug in the fistula during the rial.

EFFECT OF HEAT TREATMENT OF MILK UPON THE FREE ACID, TOTAL ACID AND HYDROGEN-ION CONCENTRATION IN THE STOMACH OF THE CALF

Standard clinical methods were adopted in this experiment with calves. These methods were supplemented (a) by palpation through the fistula, and (b) by removing and weighing the curd at definite intervals after the feeding of the test meal. Part of the analyses during typical trials are shown in figures 2, 3 and 4 because they present interesting information concerning the physiology of digestion in calves.

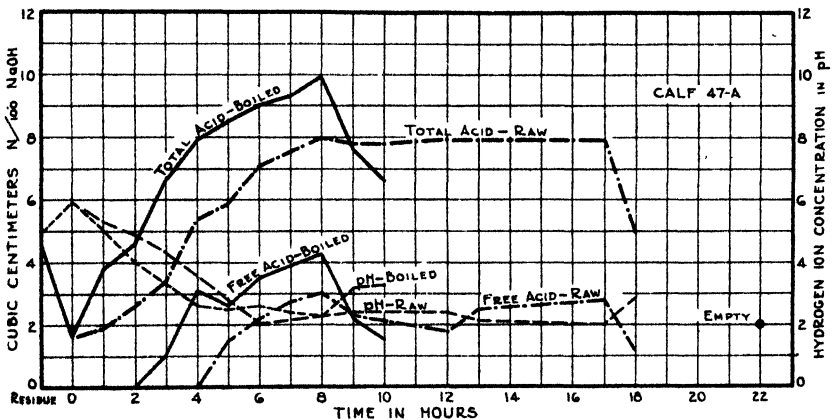


FIG. 3. EFFECT OF RAW AND BOILED MILK UPON TOTAL ACID, FREE ACID, AND HYDROGEN-ION CONCENTRATION OF GASTRIC JUICE AND EVACUATION TIME OF STOMACH.

The results of feeding raw and boiled milk on the total acidity of the gastric juice in calf 47-A are shown in figure 2. It will be noticed that the curves for boiled milk are much the same. The peak in total acid is reached near the eighth hour. From then on the acidity drops rapidly until digestion is complete. Occasionally there are sharp rises as shown in curve No. 1 with boiled milk and curve No. 1 for raw milk. These fluctuations are due to some physiological stimulation beyond the control of the investigator. The curves on raw milk, although different, follow the curves for boiled milk until the eighth hour when the curves for raw milk tend to flatten out rather than drop abruptly.

Data on free acid and hydrogen-ion concentration, as well as total acidity, are shown in figure 3. Again the form of the curves is much the same for raw and boiled milk except that the latter curves are steeper.

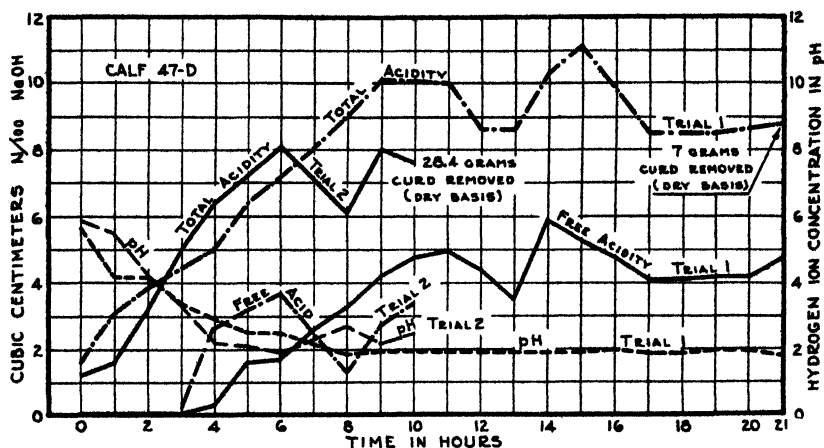


FIG. 4. EFFECTS OF PASTEURIZED MILK UPON GASTRIC SECRETION AND EVACUATION TIME OF STOMACH.

These two tests (Fig. 3) were run on the same calf, 47-A, and were about six days apart.

The results of two tests on pasteurized milk fed to calf 47-A are shown in figure 4. One test was discontinued at the end of 10 hours and the curd removed and weighed. The other test was terminated in 21 hours. A few grams of curd still remained. The tests are too few to be conclusive. However, they indicate that pasteurized milk leaves the stomach in about the same time as raw milk. The acidity curves are much the same. Irregularities in the curves may be due to regurgitation of juices from the duodenum.

From the data which has been presented, and other incomplete trials, it seems that the acidity of the gastric juice is fairly uniform regardless of the processing of the milk. Of course the acidity curves vary but this is likely due to the individuality of the calf. Boiled milk and autoclaved milk leave the stomach faster than raw milk, not because the gastric juice varied in acidity when these milks were fed but more likely because the effect of heat lowers the curd tension and thus allows the gastric juice more curd surface to attack due to the presence of smaller curd masses. Raw milk coagulates in the abomasum of the calf in from one to ten minutes. Boiled milk usually requires about 8 minutes to show the first signs of flocculation and about 15 minutes to really whey off. The minimum time required to coagulate boiled milk was 2 minutes. On the other hand one sample of boiled milk and one sample of autoclaved milk resembled a thick cream soup two hours after ingestion.

One of the authors (F.N.M.) ran a series of trials on himself with aliquot

samples of raw and heat treated milk. Three hundred cubic centimeter portions were used for each trial. Results typical of those secured after drinking boiled milk were obtained when autoclaved milk was drunk. Though the emptying time of the stomach is shorter in the case of man than with calves the acidity curves obtained for each species follow the same general trend. The free and total acid seem to increase at about the same rate in all instances. But the more rapid drop in acidity in the case of the boiled milk would indicate that the heated milk is more easily liquefied, resulting in an early diminution in gastric secretion and rise in pH.

#### SUMMARY

1. Using a modification of the Hill technique it was found that boiling of skim milk for 3 minutes in an open container on an oil bath lowered the curd tension about 80 per cent. Pasteurizing skim milk at 142° F. for 30 minutes in a vat pasteurizer reduced the curd tension about 20 per cent. Autoclaving at 242° F. for 15 minutes reduced the curd tension to zero. Plain condensed skim milk when diluted to the specific gravity of average skim milk gave a curd tension near that of boiled milk.

2. The rate at which these milks leave the stomach was then studied by palpating the curd mass in the abomasum through the wall of the rumen. Two liters of raw skim milk usually left the abomasum in about 12 to 18 hours when fed by stomach tube. The evacuation time of the same quantity of boiled milk was usually 8 to 12 hours. Equivalent amounts of boiled and autoclaved milk remained in the stomach about the same length of time.

3. At varying intervals of time, curd was removed through a gastric fistula from the stomach of calves. The data indicate that equal amounts of raw and boiled milk are liquefied at much the same rate during the first 3 to 6 hours. After this the boiled milk liquefies at a faster rate.

4. Measurements of the total acid, free acid and hydrogen-ion concentration were made upon the gastric contents of calves which had been fed test meals of equivalent quantities of raw and heat treated milk. Boiled milk and autoclaved milk leave the stomach more quickly than raw milk. This is not because the gastric juice varies in acidity when these milks were fed, but more likely because the effect of heat lowers the curd tension and thus permits the curd to break up more easily. The breaking up of the curd furnishes a greater surface for the gastric juice to attack.

5. Raw milk coagulates in the stomach of the calf in from 1 to 10 minutes. Boiled and autoclaved milk coagulate more slowly in the stomach—usually requiring 8 to 15 minutes.

6. The variability of the results obtained with the same type of test meal fed under as nearly identical conditions as possible are probably due to fatigue, individuality and the state of health of the animal.

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# THE GERMICIDAL EFFICIENCY OF LYE AND CHLORINE SOLUTIONS FOR THE STERILIZATION OF MILKING MACHINES AND CREAM SEPARATORS<sup>1</sup>

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## INTRODUCTION

The wide-spread use of chlorine compounds and the more recent introduction of alkali compounds for the disinfection of dairy equipment present a question as to their relative efficiencies under dairy farm conditions. In this work an attempt has been made to compare the results obtained with lye (sodium hydroxide) and chlorine (sodium hypochlorite) solutions used in the sterilization of milking machines and cream separators.

## EXPERIMENTS WITH MILKING MACHINES

*Procedure:* To insure a high initial bacterial contamination, 3 gallons of skim milk heavily inoculated with *Escherichia coli* were drawn through each of 3 new milking machines. This was followed by 3 gallons of cold, water, after which the teat cups and tubes were left at room temperature for a period of 48 hours before exposure to one of the disinfectant solutions.

The bacteriological condition of the respective milking machines before and after treatment with disinfectant solutions was determined by drawing 3 liters of sterile water through each unit from a sterile, imitation udder made of tin. The plate count per cc. of this water was used as an index to the sanitary condition of the machine.

*Solution Rack Method.* Sixteen trials were run in which the germicidal efficiencies of lye and chlorine solutions were compared. In each trial 3 solution racks were used, the reservoirs of which contained water, lye solution, and chlorine solution, respectively.

After samples had been taken for the purpose of determining the initial contamination, the teat cups were attached to the solution racks and filled with the respective solutions. At the end of 48 hours the solutions were drained from the teat cups and tubes, and the bacteriological condition of the machines determined.

In 10 of the 16 trials comparisons were made between the germicidal

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efficiency of 0.5 per cent lye solution and chlorine solution containing approximately 200 parts per million (p.p.m.) available chlorine, and in the 6 remaining trials between 0.3 per cent lye solution and chlorine solution containing 100 p.p.m. available chlorine. The results of these studies are summarized in table 1.

TABLE 1

*A comparison of the germicidal efficiency of lye and chlorine solutions when used with milking machines. (Solution rack method)*

	DISINFECTANT USED					
	Water		Lye		Chlorine	
Number of trials	10	6	10	6	10	6
Initial concentration <sup>1</sup>			0.510	0.322	193	101
Final concentration			0.484	0.284	1.5	0
Per cent loss in concentration			5.09	11.8	99.2	100
Initial bacterial count <sup>1</sup>	119,862	165,816	363,030	200,233	152,925	90,750
Final bacterial count <sup>2</sup>	768,300	475,000	2,098	506	1,133	726
Per cent increase or decrease in bacterial count	+ 540	+ 186.4	- 99.42	- 99.74	- 99.25	- 99.19

<sup>1</sup> Concentrations of the lye solutions are expressed as percentage of sodium hydroxide, and of the chlorine solutions as parts per million (p.p.m.) of available chlorine. The values given in the table are averages of the results of all of the replicate experiments.

<sup>2</sup> The initial bacterial count was determined by making plate counts on 3 liters of water which had been passed through one of the contaminated machines before treatment. The final bacterial count is represented by the plate count per cc of 3 liters of water which had been passed through the machine immediately after treatment with disinfectant solution or with water in the case of the control. The bacterial counts, therefore, merely serve as arbitrary indexes of the sanitary condition of the machine before and after treatment. The values given in the table are averages of the results of all of the replicate experiments.

The germ-killing efficiency of the chlorine solutions containing approximately 100 and 200 p.p.m. available chlorine proved to be nearly as high as that of the lye solutions. Although the available chlorine was almost entirely depleted at the end of 48 hours, over 99 per cent of the bacteria had been destroyed in all cases.

*Immersion Method.* Three 8-gallon crocks were filled with water, chlorine solution, and lye solution respectively. Except for the fact that the teat cups and tubes were immersed in the respective crocks, the procedure was identical with that in the preceding trials. In a series of 4 experiments it was found that the average germicidal efficiencies of the chlorine and lye solutions were 96.97 and 99.50 per cent, respectively. The bacterial counts of the check machines, which were treated only with water, showed an average increase of 135.9 per cent for the 4 experiments.

At the conclusion of these trials the chlorine solutions, the average

initial concentration of which was 215 p.p.m., were completely depleted of chlorine. The lye solutions, on the other hand, decreased from an average initial concentration of 0.52 to 0.43 per cent sodium hydroxide at the end of the experiments.

The results of 6 subsequent experiments, carried out under dairy farm conditions, clearly demonstrated the loss in concentration of chlorine solutions when the immersion method was used. The chlorine solutions when placed in the crocks gave an average test of 195 p.p.m., and after 24 and 48 hours use only 98 and 38 p.p.m., respectively. A 0.56 per cent lye solution under the same conditions contained 0.44 per cent sodium hydroxide at the end of one week. It is doubtful if the period of utility of chlorine solutions extends beyond 48 hours when the immersion method of sterilizing milking machine parts is used.

#### EXPERIMENTS WITH CREAM SEPARATORS

*Procedure:* Sterilized parts of the 3 separators were assembled and the machines uniformly contaminated with a mixed flora of microorganisms by pouring through 6 gallons of specially prepared skim milk. One of the separators was designated, for the purpose of a single experiment, as the check machine and was rinsed with 3 liters of sterile water. Bacterial plate counts on this water were accepted as a general index of the initial contamination of the separators. The other separators were then rinsed with 3 liters of one of the various concentrations of disinfectant solutions employed. After treatment, each separator, including the check machine, was flushed again with 3 liters of sterile water. The plate counts per cc. on these samples of water were used as relative indexes of the sterilizing action of the disinfecting solution employed, and, in the case of the check machine, of the mechanical washing effect. Although the values given in the tables are averages of all of the replicate experiments, they reflect quite satisfactorily the general trend of the results of individual experiments. The concentrations of the various disinfectant solutions were determined before and after use.

*Comparison of lye (0.5 per cent) and chlorine (200 p.p.m.) solutions.* It will be observed in table 2 that the lye solutions lost 14.72 per cent of their alkalinity, whereas the values for available chlorine decreased 58.13 per cent. This loss in concentration of available chlorine should not be regarded necessarily as detrimental, providing the solution was not so depleted that it had lost its germicidal power entirely. A chlorine solution for example which shows the same test before and after use apparently has yielded none of its chlorine to the process of disinfection.

The reduction in bacterial count reflects some interesting points. Under the conditions imposed by the experimental procedure about 82 per cent

TABLE 2  
*Comparison of lye (0.5 per cent) and chlorine (200 p.p.m.) solutions for the sterilization of cream separators*

	WATER	LYE, APPROXI- MATELY 0.5 PER CENT	CHLORINE, APPROXI- MATELY 200 P.P.M.
Number of trials	12	12	12
Initial concentration*		0.489	203
Final concentration		0.417	85
Per cent loss in concentration		14.72	58.13
Initial bacterial count*	3,512,727	3,512,727	3,512,727
Final bacterial count*	644,000	175,283	19
Per cent reduction in bacterial count	81.66	95.01	99.99

\* See footnotes table 1.

of the organisms were removed by water irrespective of the bactericidal power of the solution. The percentage of organisms removed mechanically, however, varies between wide extremes. Experiments not included in this paper were specially designed to evaluate this point. It was found that from 2 to 45 per cent of the organisms were removed by rinsing. The significance of the 82 per cent reported in table 2 is subject to question.

The average bacterial count of the machines after they had been treated with 0.5 per cent lye was 175,283, whereas the machines treated with chlorine solutions containing 200 p.p.m. gave an average count of only 19. (The results of the individual experiments, not given in the table, show that the machines treated with chlorine gave counts of 0 in 10 of the 12 trials, whereas the machines treated with lye solution gave bacterial counts ranging from 2700 to 1,700,00 per cc.).

The consistent failure of the 0.5 per cent lye solutions to sterilize the machine (bacterial count = 0 per cc.), and the consistently low bacterial counts obtained when the separators were treated with chlorine suggests that the rapidity of action of a disinfectant should be considered in selecting a sterilizing agent for cream separators. The superiority of the chlorine may be due to a greater rapidity of action than that of the lye solutions.

*The effect of various concentrations of chlorine disinfectant solutions.* In 7 replicate experiments using 4 separators in each, comparisons were made of the relative germicidal properties of chlorine solutions containing approximately 200, 100, and 50 p.p.m. available chlorine.

It will be observed in table 3 that the chlorine solutions originally testing approximately 200, 100, and 50 p.p.m. lost in concentration 53.96, 58.42, and 82.00 per cent respectively, and further, that the per cent reductions in bacterial counts were 99.99, 99.89, and 97.70. The solutions originally testing 50 p.p.m. available chlorine were depleted during use

TABLE 3

*Comparison of chlorine solutions containing approximately 200, 100, and 50 parts per million available chlorine for the sterilization of cream separators*

	WATER	CHLORINE		
		approx- imately 200 p.p.m.	approx- imately 100 p.p.m.	approx- imately 50 p.p.m.
Number of trials	7	7	7	7
Initial concentration*		202	101	50
Final concentration*		93	42	9
Per cent loss in concentra- tion		53.96	58.42	82.00
Initial bacterial count*	2,137,142	2,137,142	2,137,142	2,137,142
Final bacterial count*	596,142	9	2,323	49,016
Per cent reduction in bac- terial count	72.10	99.99	99.89	97.70

\* See footnotes table 1.

to a point of questionable germicidal value (9 p.p.m.). The results of these experiments indicate that rinsing a separator with a chlorine solution testing approximately 200 p.p.m. gives satisfactory results from the standpoint of the destruction of bacteria. The use of solutions originally containing 100 p.p.m. or less available chlorine is likely to lead to unsatisfactory results.

*The effect of various concentrations of lye solutions.* In 8 replicate experiments comparisons were made of the relative germicidal properties of lye solutions originally testing approximately 1.00, 0.75, 0.50 per cent.

The results showed the percentage loss of the original concentrations of these solutions to be 15.38, 9.33, and 17.31 respectively. The percentages of reduction in the bacterial counts were 99.45, 99.73, and 99.39 respectively. These results confirm the observations of Myers<sup>1</sup> and McCulloch<sup>2</sup> who showed that the concentration of lye solutions (within certain limits) is not an important factor in its germicidal activity.

*The effect of the time of action of lye solutions.* The results of previous experiments suggested that in pouring a relatively small volume (3 liters) of 0.5 per cent lye solution through a separator, the time of exposure was insufficient for effective sterilization. Obviously, the time of exposure could be increased by using larger volumes of the lye solution.

When the times of contact were 0.5, 1.0, 2.0, and 4.0 minutes the aver-

<sup>1</sup> Myers, B. P., 1929. The germicidal properties of alkaline washing powder, with special reference to the influence of hydroxyl-ion concentration, buffer index, and osmotic pressure. *Jour. Agr. Res.*, 38: 521-563.

<sup>2</sup> McCulloch, E. C., 1933. The germicidal efficiency of sodium hydroxide. *Jour. Bact.* 25: 469-493.

age percentages of bacteria destroyed or removed were 98.67, 99.69, 99.96, and 99.99 respectively. The average losses in concentration of the lye solution for the corresponding time intervals were 41.0, 24.8, 12.2, and 7.4 per cent respectively. Although the details of the data are not presented in tabular form, they suggest that when lye solution is used in sufficient volume to require from 2 to 4 minutes to run through the machine, adequate destruction or removal of bacteria may be expected.

*Effect of a 0.5 per cent lye solution on separator discs.* While it may be possible to bring about adequate destruction of bacteria in a cream separator by the use of a large volume of lye solution, such practice is not to be recommended. It was observed that following the continued use of lye solution for the disinfection of cream separators corrosive action resulted. Accordingly experiments were planned to study the effect of prolonged exposure of separator discs to lye solution.



The corrosive action of 0.5 per cent lye solution on new separator discs.

The periods of exposure were as follows: No. 1—control not exposed, No. 2—6 hours, No. 3—10 hours, No. 4—13 hours, No. 5—15 hours.

In each of two experiments, a series of 12 new separator discs were immersed at room temperatures in a 0.5 per cent lye solution. Observations made at frequent intervals up to 15 hours showed appreciable corrosive action after 6 hours' exposure. Virtually all of the tinning on both sets of discs was removed after 15 hours' exposure. The illustration shows one series of discs which had been immersed in a 0.5 per cent lye solution for 6, 10, 13, and 15 hours respectively. Similar results were obtained with the other series of discs.

#### SUMMARY

Milking machine tubing and teat-cups may be sterilized effectively either with chlorine solutions testing 100 p.p.m. available chlorine, or 0.3

per cent lye solutions, providing the solution rack method is used. When the immersion method is employed, the chlorine solutions are less satisfactory than the lye solutions.

Chlorine solutions proved to be a more effective sterilizing agent than lye when used as a disinfectant rinse for separators. The results indicate that chlorine solutions employed for this purpose should contain at least 200 p.p.m. available chlorine in order to insure effective sterilizing action. Little difference was noted in the bacteriological efficiencies of lye solutions testing 1.00, 0.75, and 0.50 per cent, respectively. Since exposure of separator discs to a 0.5 per cent lye solution tends eventually to corrode the surfaces its use cannot be recommended for sterilizing cream separators.



## SEASONAL VARIATIONS IN THE LIPASE CONTENT OF MILK

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In the commercial production of raw cream, there occurs at certain seasons of the year a bitter flavor, accompanied by an odor resembling butyric acid; by an increase in titratable acidity; and by a peculiar, burning sensation at the back of the tongue when the cream is swallowed. The defect usually develops only after the cream has been held in the raw state for from 24 to 48 hours, although occasionally it develops much sooner. Pasteurization always checks the development of the defect, and it usually does not appear except in those cases where the cream must be held in the raw state for some time prior to pasteurization. Skim milk does not show the defect, even though the cream taken from the skim milk becomes very bitter. Since these facts indicate the possibility of fat hydrolysis, an investigation was undertaken to determine the extent to which lipase is present in mixed milk as received at commercial cream plants in New York State, with special reference to seasonal variations.

### LITERATURE REVIEW

Although several reports of investigations bearing on the question of the lipase content of both human and cow's milk appeared in the literature prior to 1909 (see references 1 to 17 inclusive), Maass in that year presented the first really convincing evidence that cow's milk contains a true lipase capable of hydrolyzing milk fat (18). Using formalin as a preservative and sterilized cream as a substrate, he found that the addition of raw milk or cream caused an increase in titratable acidity after incubation at 35–38° C., and that this increase was proportional to the amount of inoculum, a fact which is characteristic of an enzymatic rather than of a bacterial process. He reported that the amount of the enzyme present in milk increases at the end of the lactation period. Unfortunately, this excellent work of Maass seems to have been overlooked by most of the subsequent workers.

Rogers and his associates (21, 24) reported some work in 1912 and 1913 which seemed to show the presence of lipase in milk, although their work was weakened by the fact that they used esters other than natural milk fat as a substrate. Palmer (32) showed quite conclusively that milk from cows in advanced lactation contains a true lipase, although he failed to demonstrate it in milk from cows not near the end of lactation. (29, 30, 31).

Although other investigators reported work bearing on the subject

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(references 19, 20, 22, 23, 25, 26, 27, 28), the first work of real significance after that of Maass was reported by Rice and Markley (33), who in 1922 showed that normal milk *does* contain a very active lipase, which sets fatty acids free from milk fat in the absence of bacterial growth. They used boiled cream as a substrate, preserving it by saturating with sucrose, and titrating with alkali to determine increase in acidity. This is the same method used by Maass in 1909 except for the substitution of sucrose for formalin as a preservative.

Beumer (34) and Virtanen (35) reported failure to demonstrate true lipase in cow's milk, but its presence was confirmed by Rice (38), Koestler (36), Koestler, Roadhouse and Lörtscher (37), Roadhouse and Koestler (39) Nair (40), and Davies (42).

In 1932 Dorner and Widmer (41) noted that homogenization of raw milk causes it to become rancid very rapidly, in from one-fourth to two hours, with a bitter flavor and an increase in acidity. This is in accord with the observation of Beatty (25) and of Frazier and Walsh (52) that lipase acts only on the surface of the fat, so that homogenization, by increasing the fat surface, would enable the action to proceed at a more rapid rate. The findings of Dorner and Widmer have since been confirmed in this country by Halloran and Trout (43), Sharp and de Tomasi (45), Trout (46, 47, 54), Doan (50), Doan and Minster (51), and Ramsey and Tracy (53).

The observation of Maass (1909) that the lipolytic activity in milk increases as the lactation period advances has been confirmed by Palmer (32), Leitch (44), Trout (46, 47), Bailey (48) and Csiszar (49). Csiszar (49) notes that lipolytic activity in milk is much more prevalent in winter than in summer.

#### EXPERIMENTAL

In this investigation, the method that has been used for studying quantitatively the lipase content of milk has differed from those previously used in one important respect. Instead of adding the milk being studied to sterilized cream, the milk was separated (under strictly commercial conditions); the cream so obtained was standardized to a fat content of 40-42 per cent; and fat hydrolysis in this raw cream was followed by titration of the fatty acids set free, using sodium hydroxide and phenolphthalein. These titrations were recorded as the cubic centimeters of tenth-normal sodium hydroxide required to bring nine cubic centimeters of the cream to the phenolphthalein end-point. If the decimal point in these titrations is moved one place to the left, the result will be equivalent to the common method of expressing titratable acidity as "percentage of lactic acid."

By means of this method of investigation, the substrate in which the lipase acted was raw cream separated from the milk being studied. Such a method of procedure has three distinct advantages:

1. The concentration of lipase in the reaction mixture is the highest that can be obtained, there being no dilution by the addition of sterile cream which contains no lipase. Differences that may exist in the lipase content of the milk are therefore more easily measured.

2. The fat exists in the substrate in a normal condition, unchanged by heat.

3. The conditions under which the activity of the enzyme is studied can be exactly like those under which raw cream is produced commercially, which is a distinct advantage because one of the dairy products in which lipolytic activity is most serious is commercial sweet cream which must be held for some hours or days prior to pasteurization.

Three series of data are available for study. The first of these series consists of reports, by a retail milk distributor in the New York Metropolitan area, of the acidity and flavor of shipments of raw cream produced by a small creamery throughout the fall and winter of 1930-1931. The exact conditions under which the cream was produced are not known to the writers, nor is it known what disposition was made of the cream which was defective. The data are taken from strictly routine commercial reports, none of the people involved in the production or inspection of the cream having any knowledge that any problem of this nature was being studied. The data are available to the writers because our organization had been active as an intermediary in the sale of the cream, and they are included because they show very well the seasonal nature of the difficulty; because they bring out the close correlation between acidity and flavor; and because they offer a very clear-cut substantiation, from a strictly commercial standpoint, of the data included in the other two series of experiments, which were conducted under more carefully controlled but also under somewhat more artificial conditions.

The words used on the inspection reports to describe the defective flavor of the cream were "bitter," "strong," "old" and "poor." The results are given in Table 1.

TABLE 1  
*Monthly variations in titratable acidity and percentage of poor-flavored shipments*

MONTH	CC. OF N/10 NaOH TO TITRATE 9 CC. OF CREAM	PERCENTAGE OF SHIPMENTS REPORTED AS HAVING OFF FLAVORS
October	1.20	12.7
November	1.29	16.7
December	1.34	58.4
January	1.30	40.2
February	1.27	19.0
March	1.27	6.1
April	1.24	0.0

An examination of these data shows very plainly the close correlation existing between high acidity and poor flavor. The peak for both comes in December, when one would ordinarily expect bacterial activity to be at a minimum.

The second and third experiments were carried out at cream plants where the writers had the privilege of directing procedure. In both cases the cream was separated under commercial conditions at 85°–90° F. from mixed milk from a large number of herds, and was produced to be pasteurized immediately rather than to be shipped raw. The experimental samples were taken from the cream prior to pasteurization.

In Experiment II the procedure was very simple. Each raw cream sample was held in the plant cold-room in a sterile bottle at a temperature near 40° F. for 48 hours and the acidity was then determined as follows: a 9 cc. pipette was filled to the mark with the cream to be tested, and this cream was then allowed to run into a 100 cc. beaker. The cream sticking to the inside of the pipette was rinsed into the beaker with two 9 cc portions of water, four drops of 0.5 per cent alcoholic phenolphthalein solution were added, and the mixture was titrated to a distinct but faint pink color with tenth-normal sodium hydroxide, and the number of cubic centimeters of alkali were recorded. Table 2 shows the monthly averages of these results.

TABLE 2  
*Monthly variations in production per day per dairy and in titratable acidity of cream when 48 hours old*

MONTH	NUMBER OF SAMPLES	PRODUCTION PER DAY PER DAIRY	CC N/10 NaOH PER 9 CC OF CREAM
August	20	167	1.09
September	21	159	1.22
October	21	181	1.19
November	22	167	1.40
December	21	166	1.55
January	22	182	1.65
February	20	198	1.60
March	14	216	1.08
April	9	223	1.05

In the two experiments described above the only means of preventing the activity of microorganisms was by keeping the milk and cream cold. No bacteria counts are available to show how successful this control may have been. The third experiment was planned to give more assurance of the absence of bacterial growth, and bacteria counts were made so as to check on the absence of growth.

In this experiment three 10 cc. aliquot portions of each sample of raw cream (40% fat) were saturated with powdered sucrose to prevent bacterial

growth, the method first used by Rice and Markley (33). The first sample was examined immediately, the second after 48 hours, and the third after 96 hours of digestion at 20° C. (68° F.) Each sample was diluted to 100 cc. with sterile water, 1 cc. was removed for further dilution for bacteria counts, and the remainder titrated to the phenolphthalein end point with N/10 NaOH.

In a few cases a fourth sample was prepared, bacteria counts being made immediately without addition of sucrose. These showed no immediate reduction in the numbers of bacteria due to the addition of sucrose. The sucrose did prevent growth in every case, and usually resulted in a gradual decrease in the number of bacteria.

A few bacteria counts were made on the tributyrin agar described by Anderson (55), to detect the presence of fat-splitting bacteria.

The results are summarized in Table 3, monthly averages only being shown to save space. The bacteria counts shown in Table 3 are the monthly averages of the highest of the six counts obtained for each individual sample.

The data of Table 3 are shown graphically in Figure I, with the production curve drawn inversely to show more clearly any inverse correlation between production and acidity.

TABLE 3

*Month by Month variations in production, bacteria counts, and titratable acidity of cream*

MONTH	NUMBER OF SAMPLES	PRODUCTION PER DAY PER DAIRY	HIGHEST BACTERIA COUNT	cc. N/10 NaOH PER cc.	
				fresh Cream	Cream digested 96 hours
February 1933	2	208	240,000	0.80	2.30
March	2	208	130,000	0.85	2.20
April	2	213	290,000	0.70	2.00
May	3	233	290,000	0.80	2.00
June	2	230	360,000	0.80	1.65
July	1	Milk Strike	560,000	0.80	1.60
August		Milk Strike			
September	1	186	430,000	0.80	1.90
October	2	190	230,000	0.70	2.20
November	1	186	400,000	0.90	2.80
December	1	200	120,000	0.90	3.20
January 1934	1	214	190,000	0.90	2.70
February	3	214	200,000	0.90	2.50
March	2	222	192,000	0.90	2.30

There are several significant points to be observed from an examination of Figure I. The variations in the titratable acidity of the cream immediately after skimming are too small to have any significance. Under conditions such as those in this experiment, where lipolytic activity is encouraged

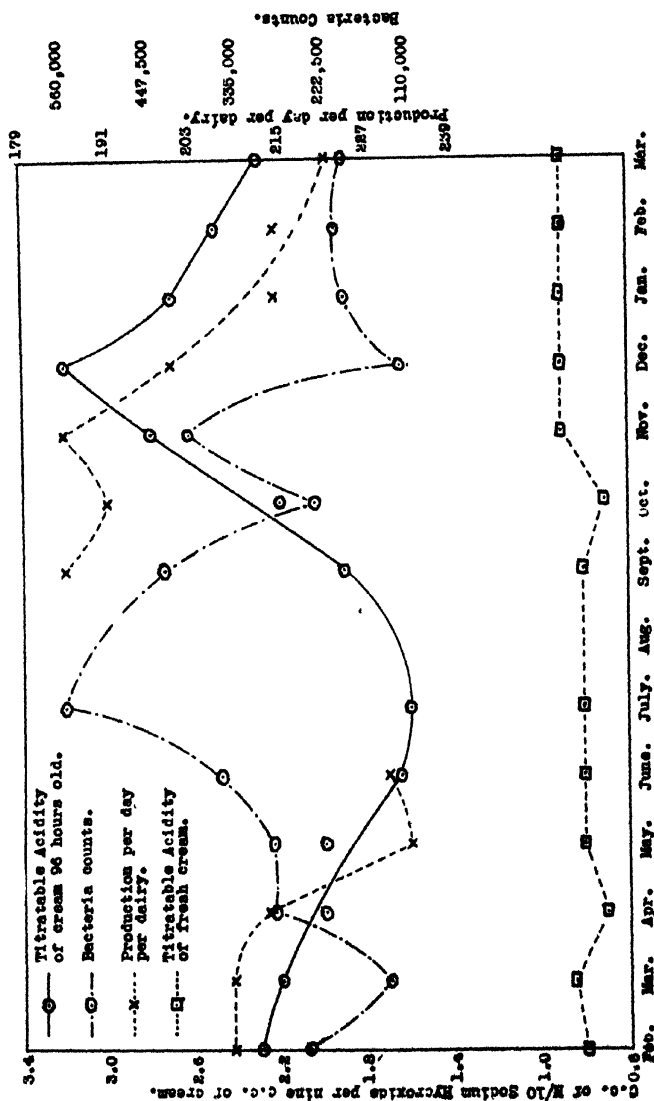


FIGURE 1. SEASONAL VARIATIONS IN TITRATABLE ACIDITY, PRODUCTION PER DAY PER DAIRY, AND BACTERIA COUNTS.

and bacterial activity is prevented, some lipolytic activity (as indicated by the increases in acidity in 96 hours) can be demonstrated in the milk throughout the entire year, although this lipolytic activity is at a minimum during early summer (June and July), while it reaches a maximum in early winter (December).

Maass (18) and others have stated that the amount of lipase in milk increases as the lactation period advances. If this be true, then there should be a close inverse correlation between the rapidity of fat hydrolysis and the "production per day per dairy," which is the average weight of milk delivered to the plant by the individual farmer.

An examination of Tables 2 and 3 and Figure I will show that there is a distinct tendency toward such an inverse correlation. (Data on production for July and August are not shown in Table 3 and Figure 1 because a milk strike among the producers affected the receipts somewhat at this plant during these two months). However, the data of both Tables 2 and 3 show production minima in September without corresponding acidity maxima. This is undoubtedly explained by the fact that these September minima in production are due to a lack of feed (pastures being poor and grain feeding not yet having started) and to the additional fact that low production due to lack of feed is not accompanied by any corresponding increase in the lipase content of the milk.

There is a second production minimum in each set of data, occurring in December in Table 2 and in November in Table 3. There is a corresponding acidity maximum in each case, but it lags one month behind the production minimum. It is believed that the explanation for this lies in the fact that some other factor, in addition to late lactation, is operative in tending to increase the lipase content of the milk, and that the summation of these two lipase-producing factors reaches a maximum one month later than the lactation minimum.

Figure III also shows the monthly averages of the highest bacteria counts obtained on each individual sample. As might be expected, the bacteria counts are somewhat erratic, but there is evident a general tendency for acidity to be low when bacteria counts are high, and vice versa. This is easily understood when we consider that the low acidity occurs in the summer months, and the high acidity in the winter months. In no case is the number of bacteria great enough to account for any increase in acidity, so that the lipase could not have been of bacterial origin.

In 1934 Anderson (55) proposed the use of standard nutrient agar with one per cent of added tributyrin to detect fat-splitting bacteria. A few counts on this agar were made, and the results are shown in Table 4. A glance at this table will show that the numbers of fat-splitting bacteria are far too low to account for the increases in acidity. Thus, in the experiment of March 20, 1934 the acidity increased from 0.90 cc. to 2.40 cc. in 96 hours,

TABLE 4  
*Titratable acidity and number of both total and fat-spitting bacteria*

DATE OF EXPERIMENT	AGE OF CREAM IN HOURS	CC. N/10 NaOH PER 5 CC. OF CREAM	BACTERIA COUNTS ON STANDARD AGAR				FAT SPLITTING BACTERIA		
			No sucrose incubated 5 days at 20° C.	Sucrose incubated 5 days at 20° C.	Sucrose incubated 2 days at 37° C.	Sucrose incubated 5 days at 20° C.	Sucrose incubated 5 days at 20° C.	Sucrose incubated 5 days at 20° C. plus 2 days at 37° C.	Sucrose incubated 2 days at 37° C.
2/13/34	0	0.90	200,000	144,000	400,000	1000			
	48	2.10		140,000	120,000				
	96	2.50		53,000	70,000	1000			
2/20/34	0	0.90		90,000	88,000				
	48	2.10		81,000	48,000				
	96	2.40		39,000	25,000	1000			
3/13/34	0	0.90	152,000	149,000	149,000	1000			
	48	2.10	84,000	45,000	45,000	3000	5000	4000	
	96	2.20		52,000	42,000	1000	12000	2000	
3/20/34	0	0.90	193,000	235,000	180,000	1000	14000	1000	
	48	2.10		318,000	112,000				
	96	2.40		133,000		2000	15000	6000	5000

while the maximum total bacteria count was 235,000 and the maximum count of fat-splitting bacteria was 15,000.

#### CONCLUSIONS

1. The increase in acidity, with accompanying bitter flavor, which occurs at certain seasons of the year in raw cream that has been held for some time at ice-box temperatures, is due to the activity of lipase.

2. This lipase is not of bacterial origin, but is secreted by the cows with the milk.

3. The amount of this lipase in milk produced in New York is at a minimum in early summer (June or July), and reaches a maximum in early winter (December or January). There is some lipase present in milk throughout the year.

4. There are two factors which govern the amount of lipase secreted with the milk. One of these is the lactation cycle, the amount of lipase increasing as lactation is prolonged. The nature of the other factor is not known.

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# THE DETERMINATION OF CURD TENSION BY THE USE OF HYDROCHLORIC ACID-PEPSIN COAGULANT<sup>1</sup>

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## INTRODUCTION

Within recent years considerable emphasis has been placed upon the curd character of milk in relation to infant feeding. The measurement of the toughness of the curd has become a useful criterion of the suitability of different milks. Buckley (2) in 1914 used dilute hydrochloric acid as a flocculating agent, and observed a marked variation in the curd character of various milks. In this manner he was able to differentiate the milks of various species of lactating animals. Alleman and Schmid (1) in 1916 devised a "tension-knife" of concentric rings to measure the toughness of milk coagula. These investigators coagulated 400 cc. of milk with 40 cc. of water at 35° C. by using 4 cc. of rennet extract. Milk having a short coagulation time was found to have a firmer curd tension. In 1923 Hill (4) described a method for the measurement of the hardness of the curd coagulated by pepsin in calcium chloride solution. He used a knife having radial blades joined to an upright slender handle. This knife is placed in a jar containing 100 cc. of milk brought to 35° C. Then 10 cc. of a coagulant consisting of three parts of a 0.6 per cent solution of 1 to 3,000 scale pepsin to one part of 37.8 per cent calcium chloride is added with slight stirring. After a ten minute interval the loop of the knife is hooked to a spring balance which registers in grams the pull necessary to cut through the curd.

Various workers (5, 8, 10) have demonstrated that coagulation is very slow in calcium-deficient milks. Calcium chloride additions, in large quantities, to milk may retard rennin coagulation, while Rona and Gabbe (9) have observed that smaller quantities may hasten the reaction.

For a period of nearly two years curd tensions were determined monthly by Hill's method on herd samples of three types of milks, which were available, namely the milk of Holstein and Jersey cows and mixed milk of Sanaan and Toggenburg goats at the United States Animal Hus-

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<sup>1</sup> The modification of the Hill Method for curd tension herein presented was the outgrowth of studies on goats' milk, the results of which are in progress of publication. The author is indebted to J. A. Gamble and A. K. Besley for their cooperation in making possible the study of curd tension measurement, to Paul E. Howe for encouragement and advice during the progress of the work and to N. R. Ellis under whose supervision the work was conducted.

bandry Experiment Farm at Beltsville, Maryland. Later when the milks were treated in various ways such as boiling, homogenizing, skimming, and calcium chloride addition, it was noticed that the calcium chloride of the coagulant exerted various undesirable influences on the curd tensions of the altered milks. For example, the analyses of the curds produced by the use of a calcium chloride-pepsin coagulant as used by Hill (4) revealed a high content of calcium, due apparently to the absorption of calcium chloride from the coagulant. It was also impossible to precipitate the albumin according to the method of the Association of Official Agricultural Chemists at the usual hydrogen-ion concentration. Hence, it seemed that if the ionic equilibrium really plays a rôle in the coagulation mechanism of milk then the calcium chloride in the coagulant must exert some undue influence. Furthermore, if the curd tension is to be a measure of gastric coagulation, then the coagulant used *in vitro* should simulate the gastric juice *in vivo*, in respect to the hydrochloric acid concentration. There appears to be no physiological reason for the presence of calcium chloride in the coagulant, and certainly not in the quantity used, for it has been shown (6, 7) that the calcium content in human gastric juice averages between 0.001 and 0.01 per cent. Therefore, the purpose of the studies described in this paper was to determine the suitability of hydrochloric acid in place of the calcium chloride in the coagulating medium.

#### EXPERIMENTAL PROCEDURE

The hydrochloric acid concentration in gastric juice (3) is held to vary from 0.2 per cent to 0.5 per cent. Therefore, a study was made of the effect of coagulants containing hydrochloric acid. Solutions were prepared by dissolving 0.45 gram of pepsin in 100 cc. of hydrochloric acid of the various concentrations of 0.2 to 0.5 per cent, inclusive. The technique used was the same as that described by Hill, including the 10 cc. amount of the coagulant. The curd tension values found were practically alike when the hydrochloric acid concentrations varied from 0.2 to 0.5 per cent. Evidently, variations of these concentrations of hydrochloric acid exert only slight changes. It was therefore decided to use 0.4 per cent acid concentration because it approximates the optimum hydrogen-ion concentration for the pepsin.

All samples of milk were studied in duplicate and when possible in triplicate at the same time comparatively using the calcium chloride pepsin coagulant described by Hill and the hydrochloric acid-pepsin coagulant outlined above.

#### RESULTS

As is shown in Table 1, the curd tensions of all three milks in the raw state obtained by the calcium chloride method (calcium chloride-pepsin

TABLE 1

*Comparison of curd tension readings of raw and treated milk as determined with the calcium chloride and hydrochloric acid coagulants and the percentage changes in the curd tensions of the treated milks compared with the corresponding raw milk curd tensions*

TREATMENT OF MILKS	NO. OF SAMPLES	Coagulant Used			
		CaCl <sub>2</sub> -pepsin		HCl-pepsin	
		In grams	In per cent change*	In grams	In per cent change*
GOAT MILK					
Raw	6	27		37	
Boiled	6	15	- 45	14	- 62
Homogenized	2	21	- 22	21	- 43
Skimmed	5	34	+ 26	43	+ 16
Skimmed and boiled	5	17	- 37	13	- 65
Calcium chloride added	12	30	+ 11	31	- 16
HOLSTEIN MILK					
Raw	5	51		53	
Boiled	5	32	- 37	15	- 72
Homogenized	2	35	- 31	29	- 45
Skimmed	5	55	+ 8	59	+ 11
Skimmed and boiled	2	29	- 43	9	- 83
Calcium chloride added	2	46	- 11	46	- 13
JERSEY MILK					
Raw	6	68		75	
Boiled	6	54	- 21	32	- 57
Homogenized	2	64	- 6	56	- 25
Skimmed	5	85	+ 25	89	+ 19
Skimmed and boiled	2	66	- 3	36	- 52
Calcium chloride added	2	70	+ 3	90	+ 20

\* Negative sign prefixed to the per cent change indicates decrease in curd tension or softening of the milk curd.

Positive sign indicates increase in curd tension or hardening of the milk curd.

coagulant) were always lower than those yielded by the acid method (hydrochloric acid-pepsin coagulant). By no means were the acid curds uniformly higher for the three milks. The modified coagulant produced curd tensions of goat milk 37 per cent higher than when the calcium chloride coagulant was used, Holstein milk 4 per cent higher, and Jersey milk 10 per cent higher.

When samples of the milks were boiled for one minute, the average curd tension by the calcium chloride method of the goat milk dropped from 27 to 15, the Holstein milk from 51 to 32, and the Jersey milk from 68 to 54. However, the acid coagulant, though yielding higher curd tensions for the raw milks, showed greater softening due to boiling of the three types of milk. The acid coagulant revealed a more marked softening of

the boiled Holstein and Jersey milks than was demonstrated by the calcium chloride coagulant. For example, the raw goat milk showed a curd tension of 37 by the modified coagulant and when boiled 14, that of Holstein milk dropped from 53 to 15, and that of the Jersey milk from 75 to 32. In terms of percentage, the degree of softening as shown by the calcium chloride coagulant is 45 per cent for goats' milk, 37 per cent for Holstein, and 21 per cent for Jersey milks, while the curd tensions of the same milks by the acid coagulant were softened 62 per cent, 72 per cent and 57 per cent, respectively.

Results on the three types of milk, when homogenized unpasteurized at a temperature of 48° C. at 4,000 pounds' pressure and then tested for the extent of the softening by the two coagulants, showed a greater drop in hardness of the curd of the raw milk due to homogenization when tested by the acid coagulant than was shown by the calcium chloride coagulant. Goat milk, when homogenized and tested by the calcium chloride coagulant, showed a decrease in its curd tension of 22 per cent, Holstein milk, 31 per cent decrease, and Jersey, 6 per cent. Yet the same milk by the acid coagulant revealed greater softening, namely, 43 per cent, 45 per cent, and 25 per cent, respectively.

All milks when skimmed had tougher curds than the corresponding whole milks. The skimmed goat, Holstein and Jersey milks gave curd tensions of 34, 55 and 85, respectively, by the calcium chloride method, while the acid method revealed higher readings for all three milks. The curd tension of the goat skimmed milk tested by the calcium chloride method increased 26 per cent, the Holstein milk, 8 per cent, and the Jersey milk, 25 per cent over the respective whole milks. On the other hand, the same milks showed increased toughness of 16, 11, and 19 per cent, respectively, when tested by the acid method.

Both coagulants indicate that the boiled skimmed milks were softened to the same extent as the boiled whole milks, but the acid coagulant apparently revealed a greater softening of the Holstein and Jersey milks than the calcium chloride coagulant. The tendencies of the acid coagulant seem to be consistent throughout. As compared to the calcium chloride coagulant, the acid coagulant resulted either in the greater hardening of the curd or in greater softening, depending on the treatment of the milk. It is possible that this reaction of the acid coagulant indicates greater sensitivity to changes in the milk.

The addition of calcium chloride to milk previous to coagulating by the two coagulants was studied for the purpose of determining the influence of calcium chloride on the curd tension. To each 100 cc. of milk, 0.5 gram of calcium chloride in the form of a 20 per cent solution was added and stirred before the addition of the coagulant. In case of the goat and

Holstein milks, the two coagulants produced identical curd tensions for the corresponding milk, indicating that the calcium chloride depressed the coagulation by the acid method to the same curd tension as the calcium chloride method, but in the case of the Jersey milk, the modified coagulant was influenced in the opposite direction, resulting in a higher curd tension.

#### DISCUSSION

The fact that the coagulant containing calcium chloride with pepsin as suggested by Hill (4) yielded lower results for the raw milks than the modified coagulant with hydrochloric acid replacing the calcium chloride evidently indicates the retarding effect due to the addition of more calcium chloride in the former coagulant. From the results obtained, it would seem that the depressant effect of the calcium chloride coagulant is not uniform because of the variable salt composition of each milk.

Evidently, heat increases the coagulation time, hence the softer curd, but the addition of calcium chloride almost causes a return to normal coagulation due apparently to the replacing of the precipitated calcium. The two coagulants show their greater disparity in the curd tensions of boiled milks where the acid coagulant produced softer curds than did the calcium chloride coagulant and yet the former showed that greater softening occurred in the boiled milks. Furthermore, the boiling of milk is known to produce a soft curd. For that reason one would question whether a drop in Jersey milk from 68 to 54 when boiled and tested by the calcium chloride coagulant can be considered marked softening when soft curds are classified by Hill to be below 30. This finding is more dubious because when tested by the modified coagulant the boiled Jersey milk is softened from 75 down to 32.

As previously mentioned, skimmed milks were consistently tougher than their corresponding whole milks. Data from an unpublished manuscript on curd tension of goat milk show statistically that the fat in the milk plays only a slight rôle in the curd solidity. The fat in milk tends mechanically to prevent the formation of a solid mass due to the interspersed enmeshment of the fat globules among the coagulated protein particles. Otherwise, the correlation factor is insignificant for the fat content. But a highly significant correlation exists between protein content and the curd tension.

Again, even in the case of skimmed milks, the acid coagulant revealed that boiling caused a greater softening of all milks, including the skimmed, than the calcium chloride coagulant. Such data seem to indicate that the calcium chloride coagulant can not be used for the determination of the curd tension of altered milks if results are to be obtained comparable to those which will approximate the coagulation within the stomach. It is

therefore felt that the acid coagulant is a truer *in vitro* representation of gastric coagulation, especially since the gastric juice contains relatively little calcium, that is, less than 0.01 per cent.

Obviously, the fact that in raw milk the curd tensions were lower when tested by the calcium chloride coagulant than when tested by the modified acid coagulant would seem to indicate the depressing effect of calcium chloride. Most likely this retarding effect of calcium chloride already present in the calcium chloride coagulant is at its maximum as is evidently shown by the results obtained when 0.5 gram more of calcium chloride is added to the milk before coagulation. The additional calcium chloride exerts no further influence on the curd tensions of the three milks by the calcium chloride coagulant. On the other hand, the acid coagulant reveals that milk supplemented with 0.5 gram of calcium chloride before adding this acid coagulant is decreased to that given by the calcium chloride before adding this acid coagulant. Obviously, the calcium chloride of the calcium chloride coagulant exerts an undesirable influence if one wishes to study the effects of various additions to milk.

#### SUMMARY

1. Excess calcium chloride as is present in the coagulant suggested by Hill which contains calcium chloride and pepsin retards coagulation and hence produces a subnormal curd tension.

2. Evidence indicates that a coagulant of 0.45 gram of pepsin per 100 cc. of 0.4 per cent hydrochloric acid produces a truer picture of the curd character simulating the gastric conditions than one which contains calcium chloride.

3. Boiling for one minute softens the curd of all milks, but not to the same degree of softness. The curd tension of the calcium chloride coagulant of boiled Jersey milk revealed only a slight softening, but did show a marked drop by the acid coagulant.

4. The effects of the various treatments of the milks in terms of percentage increase or decrease of the curd tensions were almost alike for the three kinds of raw whole milks when tested by the acid coagulant.

5. On the other hand, the calcium chloride coagulant on the same milks produced effects of a more divergent percentage increase or decrease in curd tension readings.

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# RELATION OF THE PROTEOLYTIC ENZYME ACTIVITY TO THE PROTEOLYTIC ORGANISMS FOUND IN SEPARATOR SLIME

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Several types of microorganisms are of importance in causing the deterioration of cream used for butter making, and the action of the proteolytic organisms, which correspond in a great measure to the tryptic and ereptic enzymes of animal origin and in some instances the action of pepsin-like enzymes, is manifested in the course of milk and cream hydrolysis.

In the following experiments the object has been to study the relation of the activity of the proteolytic enzymes in separator slime to the numbers of proteolytic organisms present. Separator slime, rather than the milk or cream, was used because the slime represents a composite sample of a large quantity of milk or cream. The slime was obtained from about 300 gallons of milk delivered by producers to a commercial milk plant. This milk was bought on grade basis and represented a high grade of market milk.

## METHODS OF PROCEDURE FOR THE STUDY OF THE ENZYMES

The separated slime was triturated in a mortar with a 0.7 per cent salt solution until the slime was thoroughly disintegrated, then rubbed through a coarse sieve. The salt solution represented half of the mixture.

Of this mixture 10 cc. were added to 200 cc. of sterile milk and digested at 37° C. for five days with sufficient toluol to completely saturate the mixture leaving a slight excess on the surface. An analysis of the mixture was made before digestion for soluble nitrogen compounds which were not precipitated by phosphotungstic acid and by a saturated solution of zinc sulphate, these results constituted the base from which the increases were determined. After five days digestion an analysis was again made. The difference between the last analysis and the first represented the increase of soluble nitrogen compounds not precipitated by phosphotungstic acid and by zinc sulphate.

Three samples of slime were taken during the first week of each month throughout the experiment of fourteen months. The separate analyses of the three periods were averaged and the result taken to represent the changes for the specified month. This procedure gave a better average representation of the bacterial count and enzymatic activity.

The total bacterial count was secured by plating known quantities of slime in duplicate upon beef infusion agar prepared from dehydrated

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Bacto beef; counts were made after five days incubation at 21° C. The proteolytic count was secured by plating known quantities of slime upon agar containing 10 per cent milk and incubation was for five days at 21° C. Colonies which showed definite clearing were counted as proteolytic, doubtful colonies were transferred into diluted litmus milk diluted 50 per cent with distilled water, and proteolysis was recorded if present after ten days incubation at 21° C.

Table 1 gives the results of the analysis for each month. The total and proteolytic bacterial counts are expressed as the logarithm of the number in one gram of slim mixture. The nitrogen is expressed as percentage of the total nitrogen.

TABLE 1

*Proteolytic enzymatic activity of separator slime as influenced by the month of the year and its bacterial content*

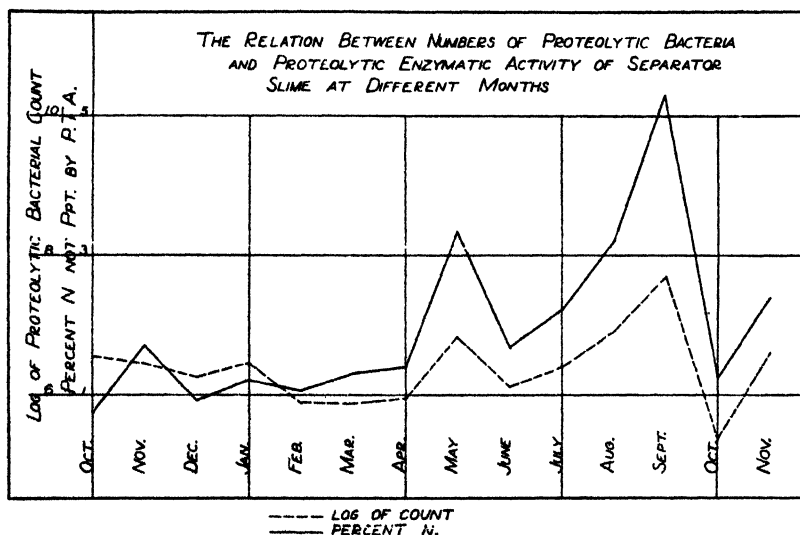
LOGARITHM OF BACTERIAL COUNT :			PER CENT NITROGEN NO1 PRECIPITATED BY :		Peptones
Date	Total bacteria	Proteolytic bacteria	Phospho tungstic acid	Zinc sulphate	
October	7.95	6.56	0.75	1.16	0.41
November	8.00	6.45	1.71	1.78	0.07
December	7.56	6.26	0.85	1.49	0.64
January	7.78	6.46	1.22	2.23	1.01
February	7.18	5.90	1.06	1.14	0.08
March	7.26	5.87	1.31	1.53	0.22
April	7.52	5.95	1.40	1.61	0.21
May	8.23	6.83	3.36	5.00	1.65
June	9.00	6.11	1.68	4.55	2.87
July	7.66	6.42	2.24	3.93	1.69
August	7.82	6.93	3.20	5.04	1.84
September	8.59	7.70	5.31	6.80	1.49
October	7.04	5.38	1.25	1.44	0.19
November	7.89	6.61	2.40	2.76	0.36

In the study of Table 1 it is well to consider the relation of the total bacterial count and the count of the proteolytic types of bacteria. The average total count of bacteria for the winter months, that is: October, November, December, January, February, March and April, was 37.59 millions or a logarithm of 7.57. For the five summer months, May, June, July, August and September, the total average bacterial count was 186 millions or a logarithm of 8.26. This shows that the slime produced in summer months contained 148.4 millions more bacteria than the slime produced in winter months. By comparing the average count for the winter months with the average count for the summer months we find the number of bacteria for the summer months was 4.94 times that of the winter months.

If we consider the proteolytic types of bacteria a similar relation exists for the summer and winter months, the total average count of the proteolytic types for the winter months was 1.63 million corresponding to a logarithm of 6.21 while for the summer months the total average was 6.31 millions corresponding to a logarithm of 6.80. The summer months contained 4.68 million more bacteria than the winter months or 3.87 times more for summer than for the winter months.

Of the total bacterial count only approximately 5 per cent appeared to be of the proteolytic type. This is true for the summer months as well as for the winter months.

The graph shows the relation between the average proteolytic count for the month and the average percentage of nitrogen not precipitated by phosphotungstic acid for the month.



In discussing the proteolytic action of the bacterial enzymes on the proteins of milk we are taking into consideration only the proteolytic types of bacteria. The hydrolyzed products produced by the proteolytic enzymes of these organisms are expressed as nitrogen compounds not precipitated by phosphotungstic acid and nitrogen compounds not precipitated by saturated solution of zinc sulphate. The relation of these two fractions gives an index as to the character of the enzymes produced by the respective organisms. The statement formerly made by us, (1), (2) corroborated by H. von Euler (34), W. Grassman (4), and Franz Fuhrman (5), that most bacterial enzymes are of the tryptic and ereptic types. The tryptic type hydrolyzes the proteins to the peptone and dipeptid state, and the ereptic

type hydrolyzes the dipeptids into amino acids. The amino acids formed appear in the fraction not precipitated by phosphotungstic acid, the dipeptids, and polypeptids (peptones) together with the amino acids appear in the fraction not precipitated by zinc sulphate, therefore, the difference of the nitrogen compounds not precipitated by zinc sulphate and phosphotungstic acid represent the peptones. This gives at least two well defined fractions of nitrogen compounds. In addition to the amino acids not precipitated by phosphotungstic acid there are some soluble compounds of nitrogen not amino acids; these same soluble compounds also appear in the nitrogen compounds not precipitated by zinc sulphate.

If we compare the different soluble compounds produced by the bacterial enzymes on milk proteins during the winter months with that of summer months we find that the ratio is 1.0 to 2.12 for nitrogen not precipitated by phosphotungstic acid, and the ratio for nitrogen compounds not precipitated by zinc sulphate is 1.0 to 2.87, which means that the hydrolysis of enzymatic activity is 2 to 3 times greater for the summer months than for the winter months. Referring this change to the proteolytic bacterial count there is shown a good relation, but not proportional to the increase in bacterial count for the winter and summer months. The bacterial ratio for winter and summer months is 1.0 to 2.9. The increase of the proteolytic bacteria increased 3.87 times during the summer months while the protein hydrolysis increased approximately three times, as summarized in activity, this relative activity of bacterial enzymes is well illustrated in Table 2. The proteolytic enzymes of proteolytic bacteria vary in their our previous work (2). A comparison of the proteolytic bacteria *B. ichthyosmius*, *A. putrefaciens*, and *S. liquefaciens* illustrate the possibility that the number of proteolytic bacteria do not of necessity imply a corresponding degree of enzyme activity on the proteins of milk.

TABLE 2

*Ratio increase of bacteria and proteolytic enzymes in separator slime obtained during winter months to that of summer months*

TOTAL	BACTERIA PROTEO.	N NOT PPT BY P.T.A.	N NOT PPT BY $ZnSO_4$	PEPTONE
4.94	3.87	2.12	2.87	5.45

#### THE INACTIVATION OF THE PROTEOLYTIC ENZYMES OF SEPARATOR SLIME BY HEAT

It was deemed advisable to study the effect of heat on the activity of the proteolytic enzymes as found in separator slime. After the addition of the separator slime to the milk the mixtures were heated to temperatures

of 145° F. for 30 minutes and at 165° F. for 10 minutes, the mixtures cooled, toluol added and the mixture digested for 5 days at 37° C. Prior to pasteurization the milk was adjusted to two pH levels, 7.0 and 5.5. The per cent inactivation of the proteolytic enzymes as found in two samples of separator slime with widely different proteolytic bacteria contents is given in Table 3.

TABLE 3  
*The effect of heat on enzymatic inactivation of separator slime*

	HEATED TO 145° F. FOR 30 MINUTES PER CENT INACTIVATED AT				HEATED TO 165° F. FOR 10 MINUTES PER CENT INACTIVATED AT			
	pH 7		pH 5.5		pH 7		pH 5.5	
	N not ppt by P.T.A.	N not ppt by ZnSO <sub>4</sub>	N not ppt by P.T.A.	N not ppt by ZnSO <sub>4</sub>	N not ppt by P.T.A.	N not ppt by ZnSO <sub>4</sub>	N not ppt by P.T.A.	N not ppt by ZnSO <sub>4</sub>
Sample I	73	78	77	60	72	71	74	72
Sample II	67	47	70	64	66	69	61	67

Sample I had a proteolytic bacterial count of 40 million per cc. while Sample II had a proteolytic bacterial count of 2 million or in terms of logarithms respectively 7.6 and 6.3.

In this experiment the effect of pH 7 and pH 5.5 was also studied, the results of which are given in table 4.

TABLE 4  
*The effect of acidity of substrate upon the enzymes found in separator slime*

	AT pH 7		AT pH 5.5		LOGARITHM OF PROTEOLYTIC BAC- TERIA COUNT
	N not ppt by P.T.A.	N not ppt by ZnSO <sub>4</sub>	N not ppt by P.T.A.	N not ppt by ZnSO <sub>4</sub>	
Sample I	3.23	4.59	1.57	2.88	7.6
Sample II	1.89	2.46	1.15	3.19	6.3

As to the relation of the bacterial count to the enzymatic activity we find that the proteolytic activity is much greater for Sample I than for Sample II, the latter containing a relatively smaller number of bacteria. The effect of pH on bacterial enzyme action is consistent with our former work on the proteolytic action of the enzymes of specific organisms, that is, the activity, in general, is greater at pH 7 than at pH 5.5.

The inactivation by heat, whether for 145° F. for 30 minutes or at 165° F. for 10 minutes is approximately the same, which agrees with our previous work on the enzymes of *B. ichthyosmius* and *A. putrefaciens*. The killing effect of heat depends on two factors, temperature and time. The inactivation was slightly greater for pH 7 than for pH 5.5; however,

the difference is not significant. It will also be noted that the percentage of inactivation was greater for the enzymes in Sample I than in Sample II, probably due to the different types of bacteria the enzymes of which are less sensitive to heat. Approximately 30 per cent of the activity remained after heating the separator slime to the temperature and time, as used in the experiment. This corroborates the statements frequently made that pasteurization does not destroy all the enzymatic action which contributes to the deterioration of dairy products.

#### SUMMARY

1. The proteolytic enzymatic activity of separator slime was greater during the summer months than during the winter months.

2. There exists a relation between the numbers of proteolytic bacteria in separator slime and its proteolytic enzymatic activity.

3. Approximately 70 per cent of the proteolytic enzymatic activity of separator slime was inactivated at 145° F. for 30 minutes and 165° F. for 10 minutes.

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3. EULER, H. VON. *Chemie der Enzyme*. Teil 2; Abschnitt 2, p. 271. Milan. 1927.
4. GRASSMAN, W. AND DYKERHOFF. Ueber die wirkungs-weise der Hefe-proteasen. *Ztsch. f. Physiol. chemie*, Volume 175, p. 18. 1928.
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# American Dairy Science Association Announcements

## GENERAL INFORMATION

It is possible to announce at this time the general program for the American Dairy Science Association Meeting to be held the week of June 24 at the University of Minnesota. The general committee believes that an especially attractive program has been arranged. The University of Minnesota is preparing to entertain a large percentage of the membership and their families. A full program of special entertainment features is being planned for the ladies which is guaranteed to prevent ennui. This will be announced in the May issue of the Journal.

The members are reminded that titles of papers to be presented must be in the hands of L. S. Palmer, Chairman of the Program Committee by May 1st. Abstracts must be sent with titles or very shortly thereafter if reprints of the abstracts are to be available for the members at the time of the meeting.

L. S. PALMER, *Chairman*  
*Program Committee*

## GENERAL PROGRAM

AMERICAN DAIRY SCIENCE ASSOCIATION  
ANNUAL MEETING, JUNE 24-27, 1935  
UNIVERSITY FARM, ST. PAUL

### JUNE 24—MONDAY

- |                 |   |
|-----------------|---|
| 9 A. M.—5 P. M. | General registration and room registration, Haecker Hall.                                       |
| 5 P. M.—9 P. M. | General Registration and room registration, Women's Dormitory, University Farm.                 |
| 2 P. M.         | Dairy Products Judging Committee (tentative) Creamery Rooms—Haecker Hall.                       |
| 7:30 P. M.      | Opening Session and Business Meeting, Auditorium, Administration Building, University Farm.     |
| 8:30 P. M.      | Social get-together for members and their families, Women's Dormitory Parlors, University Farm. |

### JUNE 25—TUESDAY

- |                 |   |
|-----------------|---|
| 8 A. M.—4 P. M. | General registration and room registration, Haecker Hall.   |
| 8 A. M.—9 A. M. | Section Committee Meetings (Rooms will be announced at opening meeting Monday evening).                                       |
| 9 A. M.—12 noon | Joint Symposium "Improving the Germ Plasm of Domestic Plants and Animals," Auditorium, Administration Bldg., University Farm. |

This symposium will be under the auspices of the following organizations:

Section O, American Association for the Advancement of Science  
 The American Dairy Science Association  
 The American Society of Agronomy (Corn Belt Section)  
 The American Horticultural Society (Great Plains Section)  
 The American Society of Plant Physiologists  
 The American Phytopathological Society

#### JUNE 25—TUESDAY (continued)

12 noon–1 P. M.	Lunch hour.
1 P. M.–4 P. M.	Section scientific meetings.
4 P. M.–4:30 P. M.	Section Committee meetings.
4 P. M.–4:30 P. M.	Members and their families leave for trip to Land-O-Lakes.
6 P. M.	Complimentary dinner and entertainment at Land-O-Lakes. Admission by ticket.

#### JUNE 26—WEDNESDAY

9 A. M.–12 noon	Section Scientific meetings.
12 noon–1 P. M.	Complimentary Dairy lunch.
1 P. M.–2 P. M.	Section business meetings.
2 P. M.–4:30 P. M.	Section scientific meetings.
5 P. M.	Leave for Minneapolis Automobile Club.
7 P. M.	Subscription banquet and entertainment at Minneapolis Automobile Club. (Tickets to be purchased on registration.)

#### JUNE 27—THURSDAY

9 A. M.–10 A. M.	Closing general session and business meeting, Auditorium, Administration Building, University Farm.
10 A. M.–1 P. M.	Section scientific meetings.
2 P. M.	Leave for arranged tours to cooperative and independent dairy plants in the Twin Cities and trips to farms of various breeders in the vicinity of the Twin Cities. Information regarding a number of these suggested tours and trips will be given members as they register, and the request will be made to signify definitely at that time which, if any, of these tours or trips is desired. This information will be required in order that necessary transportation can be arranged and the organizations or farms to be visited may be notified of the number of visitors to be expected.

# JOURNAL OF DAIRY SCIENCE

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## SOME PHYSICAL EFFECTS OF FREEZING UPON MILK AND CREAM\*

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Many observations on the physical effects of freezing upon pure sols have been reported and an interesting review of some of these studies is given by Jones and Gortner (3). An investigation of the effects of freezing upon milk and cream is complicated by the heterogeneous character of the material. The constituents of milk with which this study is chiefly concerned are the casein and the fat.

A hydrophilic sol which has been frozen will reprecipitate upon thawing whereas a hydrophobic sol similarly treated will precipitate when the frozen mass is melted. The casein of milk, being weakly hydrated retains its normal degree of dispersion under the usual conditions of freezing and thawing. The fact, however, has been noticed that when milk is held in the frozen state for considerable periods of time the casein gradually becomes insoluble (1, 2, 5). Evidently its hydrophilic properties are altered during storage in a frozen condition.

The fat is present in milk as an emulsion and is surrounded by adsorbed protein. If milk or cream has been frozen slowly free fat separates or oils off during thawing, especially when the thawing is conducted at high temperatures. If the product is frozen rapidly enough destruction of the fat emulsion can largely be prevented.

It is to be noted that while a destruction of the colloidal character of the caseinate system during freezing involves a considerable storage period, the effects harmful to the fat emulsion have occurred by the time the material is completely frozen.

A change in the distribution of the constituents when milk is partially frozen has been reported (6, 7, 11, 14). The results show a concentration of constituents in the unfrozen liquid which is to be expected since the solid to separate is pure ice. A condensing process utilizing this principle has

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recently been developed, the milk being concentrated by removing the frozen ice crystals in a centrifuge (10).

An extensive investigation of the effects of freezing upon many physical constants of milk and upon the distribution of the different constituents in the frozen mass has been reported by Cvitič (4). Other papers also present data upon phase distribution (7, 8, 9). In general the results show a concentration of the fat phase in the upper portion of the frozen mass, a higher percentage of protein in the middle or lower portions and the greatest concentration of lactose in the center or last frozen portion.

The effect of freezing upon milk and cream in relation to the marketability of these products has received much attention. A number of investigators have studied the use of frozen cream in ice cream manufacture and detailed directions for the successful handling and storage of frozen cream have been made available. Reports of this work may be found in the trade journals or in Chemical Abstracts.

#### EXPERIMENTAL

Samples were frozen in air in a cold room maintained at  $-16^{\circ}$  to  $-18^{\circ}$  C. ( $3.2^{\circ}$  to  $-0.4^{\circ}$  F.). Unless otherwise noted the containers used were either tins of 180 cc. capacity or Babcock cream test bottles graduated for 9 gram samples. The material in the tins froze completely in six to seven hours while that in the test bottles required only 50 to 60 minutes to freeze.

The degree of destruction of the fat emulsion in cream was measured as follows: 9 grams of cream were weighed into a 9-gram Babcock test bottle and placed in the  $-17^{\circ}$  C. ( $1.4^{\circ}$  F.) room for 24 hours. The samples were then thawed at  $40^{\circ}$  C. and water was added to bring the surface of the mixture near the highest graduation on the bottle. They were next placed in a  $40^{\circ}$  C. bath for 15 minutes, then whirled in a warm ( $40^{\circ}$  C.) Babcock tester for 30 minutes, removed and held in a cold box ( $15^{\circ}$  C.) overnight. The warming and whirling were repeated next day, after which time the length of the column of clear fat was read in the usual manner. Holding the cream cold between whirlings was found necessary to give a clear fat column. Control tests were always run on the unfrozen samples which generally showed about 0.5 to 1.0 per cent fat separation. These figures were subtracted from the readings obtained on the frozen samples. Correction was thus made for the quantity of fat which the method of testing caused to separate and for the action of homogenization in retarding the rise of very small globules into the neck of the test bottle.

Relative viscosity measurements on the frozen and thawed milks were made at  $30^{\circ}$  C. with an Ostwald type viscosimeter which measured the time required for 24 cc. of milk to flow through one of three different sized

capillary tubes. The water rate for each of the tubes was ascertained and the results are expressed as relative viscosity.

Coagulation and heat stability tests were conducted by sealing the samples in pyrex test tubes and sterilizing them at 120° C. in a glycerine bath.

SOME EFFECTS OF FREEZING UPON THE DISPERSION OF  
THE CALCIUM CASEINATE SYSTEM

Reference has been made to the gradual change of the calcium caseinate to an insoluble form when milk is held frozen. During this work a progressive decrease in the dispersed state of the casein was produced by freezing.

The effect of freezing upon the heat stability of skim milk was investigated. Data from a representative experiment are given in table 1. Skim

TABLE 1  
*Effect of freezing at -18° C. (0.4° F.) upon the heat stability of skim milk. Freezing time 6½ hours in cans)*

TIME FROZEN	TIME OF COAGULATION AT 120° C	
	9% S N F	18% S N F.
Weeks	Min	Min
Not frozen	204	102
7	215	105*
12	225*	95**
17	235**	0
33	205	

\* First distinct separation of casein.

\*\* Clear serum could be removed by thawing the frozen mass on a filter at room temperature.

milk of 9 per cent solids was unchanged in heat stability after storage for 33 weeks in a frozen condition. All the samples of this milk were used after the termination of this time, but from the appearance of the milk it was believed that its stability would have dropped to zero in a few more weeks. The milk of 18 per cent solids did not possess any stability toward heat on the 17th week.

Visible separation of the casein in both 9 per cent and 18 per cent milks began to take place early in the storage period. When the precipitated casein first appeared it redispersed during heating but as the time of storage increased more and more casein remained in an insoluble state during sterilization. The precipitated casein did not at first appear to affect the stability of the remaining colloidal particles. Separation of the casein

finally became very marked and if the frozen mass was allowed to melt on a filter, a clear filtrate could be obtained. The calcium caseinate from the 9 per cent milk which remained on the filter when the 12-week sample was thawed possessed remarkable heat stability. Mixed with water in place of serum, its heat stability was 195 minutes; and when the serum from the 18 per cent solids sample was used as a continuous phase, the calcium caseinate from the 9 per cent sample showed a stability of 366 minutes.

The effect of freezing upon the dispersed state of casein in skim milk is shown in a different way through some representative data plotted in Fig. 1. Duplicate cans of skim milk were sterilized at 120° C for different

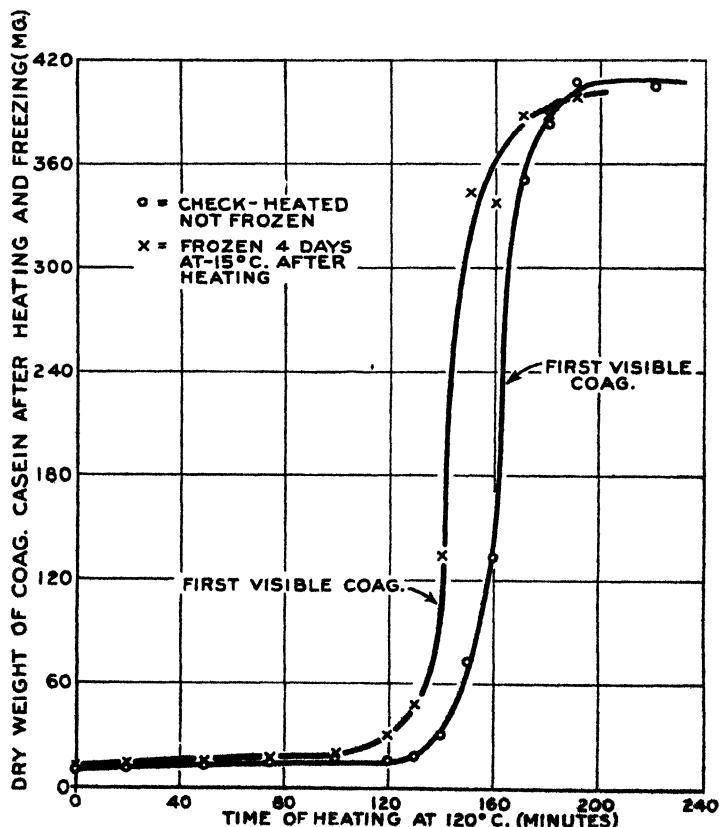


FIG. 1. THE EFFECT OF FREEZING UPON THE PRECIPITATION OF CASEIN IN SKIM MILK HEATED TO 120° C. FOR DIFFERENT LENGTHS OF TIME.

periods of time up to 220 minutes. One set of milks was frozen at -15° C. (5° F.) for four days, the other set acting as the unfrozen control. After thawing the frozen samples at room temperature 10 cc. of milk from each

can was measured into a weighed centrifuge tube. These were centrifuged at a high speed for half a hour, the supernatant liquid decanted and the precipitated casein dried and weighed. The curves of Fig. 1 are of the same type but the freezing process has caused a considerable increase in the amount of heated casein which was found precipitated. The change after a few days freezing is so slight it cannot be detected by visual examination of the sol or by comparing its heat stability before and after freezing. Since this decrease in the degree of dispersion of the casein occurred within a few days after freezing, it indicates that denaturation of the caseinate system during freezing proceeds in a very limited but progressive manner.

#### SOME EFFECTS OF FREEZING UPON THE FAT EMULSION

While freezing milk over a long period gradually produces an increase in precipitated protein its effect upon the fat phase is much more rapid.

The amount of destruction of the fat emulsion in frozen milk and cream is dependent upon several variable factors. The most important of these appear to be the freezing point of the aqueous phase, the protection afforded the emulsified fat by adsorbed protein and the size of the globules themselves.

The proportion of fat "oiling off" or separating from frozen creams of different fat percentages was measured by means of the test previously described. Large differences in the amount of fat separating after freezing were found. Generally about 25 to 50 per cent of the fat present in 20 to 40 per cent cream and 40 to 60 per cent or more of the fat present in creams over 40 per cent was freed from its normal emulsoid state by freezing. Different creams of the same fat content often showed considerable differences in the amount of fat which separated. In rare instances a cream sample was obtained in which the fat globules were apparently very well protected since only 2 or 3 per cent fat could be separated by the above test after freezing.

Two methods were employed to lower the freezing point of cream, one adding cane sugar and the other increasing the milk-solids-not-fat. Cane sugar in quantities ranging from 5 to 25 per cent added to cream before freezing was found to retard markedly the oiling off of the fat after thawing. The larger quantities of sugar almost entirely inhibited fat separation.

An increase in percentage of milk-solids-not-fat retarded the destruction of the fat emulsion in cream and a decrease in solids favored fat separation. Representative data are presented in table 2.

As the percentage of solids-not-fat of the cream was decreased the test readings for free fat increased from 5.5 to 27 per cent which, on the basis of the total fat present, corresponds to a destruction of the fat emulsion from 12 to 54 per cent. The protective action of high solids can probably

TABLE 2

*Effect of variation in solids-not-fat upon the fat separation in frozen and thawed cream thinned from cream containing 60%-63% fat. (Freezing time 60 minutes at -18° C.) Figures represent fat column readings.*

MIXTURE NUMBER	THINNING INGREDIENT	COMPOSITION WHEN FROZEN		FAT SEPARA- TION AFTER THAWING
		Fat	S N F	
		%	%	%
1	Condensed milk	45.5	12.14	5.5
2	Water and con- densed milk	46	9.81	10.5
3	Skim milk	45	4.86	15.0
4	Skim milk	40.5	4.05	15.0
5	Water	50	2.62	27.0

be attributed partly to the protection afforded by the proteïn but chiefly to the lowering of the freezing point caused by the lactose and salts.

The degree of dispersion of the fat slightly influences its capacity to undergo freezing without undue fat separation. The effect of homogenization upon the stability of the fat emulsion was studied. The data of table 3 represent the average figures from three series of experiments. Homog-

TABLE 3

*Effect of homogenization upon fat separation in cream during freezing (Freezing time 60 minutes at -18° C.) Figures represent fat column readings*

FAT SEPARATION AFTER THAWING			
FAT IN CREAM	Not homogenized	Homogenized at	
		1500 lbs	3000 lbs
%	%	%	%
10	1.4	1.1	0.7
20	7.9	5.7	3.6
30	10.5	16.1	18.5
40	25.6	22.2	24.9

enization of 10 per cent and 20 per cent creams helped to decrease fat separation during freezing but in 30 per cent and 40 per cent creams this inhibiting influence was not evident. The amount of clumping caused by homogenization of low fat creams is small but becomes much greater as the fat content of the cream is raised. The clumps are easily broken up by the freezing process and the fat emulsion is destroyed.

The results appear to indicate that the fat emulsion in ice cream remains intact for at least three reasons. The high percentage of cane sugar lowers the freezing point of the aqueous phase; homogenization of the mix inhibits fat separation and increases the amount of protein and gelatin adsorbed; and the formation of countless small ice crystals in the freezer prevent the growth of large and destructive crystals.

Interesting evidence which shows the extent to which freezing destroys the fat clumps in homogenized cream was obtained. Homogenization is known to lower to a striking degree the heat stability of cream. The lowered stability is considered to be due to the formation of fat clumps during homogenization, these clumps acting as nuclei around which coagulation may proceed (13). Data resulting from a study of the heat stability of homogenized creams before and after freezing are presented in table 4.

TABLE 4

*Effect of freezing for 24 hrs. at -18° C. upon the heat stability of cream heated to 80° C. and homogenized at 2500 lbs. pressure prior to freezing.  
(Freezing time 6½ hours in cans.)*

FAT %	TREATMENT	HEAT STABILITY AT 120° C.	
		Before freezing	After freezing
		min.	min.
10	Not homog.	122	121
	Homog.	102	120
20	Not homog.	135	137
	Homog.	75	137
30	Not homog.	142	142
	Homog.	2	146

Freezing restores to the cream the heat stability which it possessed before large fat clumps were formed by homogenization. The clumps are apparently disintegrated during freezing and cannot therefore initiate a general flocculation of the caseinate system during heating. Accordingly a homogenized cream which feathered in coffee because of excessive fat clumping could be freed of this defect by freezing. However the free fat which would oil off on the coffee after the addition of frozen cream would be undesirable.

#### PRACTICAL APPLICATION OF STUDIES ON THE EFFECTS OF FREEZING ON MILK AND CREAM

An application of the foregoing study to manufacturing problems has yielded some interesting data and the possibility of obtaining new products which may be of value either to research workers or to the industry itself.

A concentrated frozen milk (12) may be produced by pasteurizing fresh whole milk, condensing it to one-third of its weight, sealing it in containers and subsequently freezing it in an ice box maintained at about  $-17^{\circ}\text{C}$ . Such a milk, if held at a temperature below  $-13^{\circ}\text{C}$ . ( $8.6^{\circ}\text{F}$ .) may be thawed and easily reconstituted with cold water. It will yield a normal fresh milk at any time up to the 4th week of storage. This product is an excellent substitute for market milk where the latter is expensive or not easily available. It is essential that the milk be handled in equipment constructed of metals other than copper since contamination with this metal will cause a metallic flavor to develop in the product after a storage period of approximately one week.

The usual fat separation which occurs during the thawing of slowly frozen whole milk does not take place in milk condensed to a 3:1 ratio before freezing. The high solids-not-fat content of the product prevents fat separation in the milk just as it does in the creams referred to in table 2.

The denaturation of the casein during freezing is more readily noticed when the solids-not-fat content of the milk is raised. If the milk is condensed to less than one half its weight, the casein concentration is sufficiently great to produce a gel structure as denaturation in the frozen state sets in.

The relation between concentration, fat separation and protein denaturation or gel formation in frozen milks condensed to various degrees is shown by the data given in table 5. A milk evaporated to three times its

TABLE 5  
*Relation between concentration, fat separation and gel formation in frozen milk.*  
(Freezing time  $6\frac{1}{2}$  hours in cans.)

CONCENTRATION NORMAL = $\frac{\text{FAT } 4\%}{\text{SNF } 9\%}$	FAT SEPARATION		TIME TO FORM GEL AT $-15^{\circ}\text{C}$ .
	18 hrs.	2 mos.	
$\frac{1}{2}\text{ N}$ (% fat $\times 2$ )	2.0	3.0	No gel. Ppt. in 3 mos.
N	1.0	1.5	"
2 N	trace	trace	3 mos.
3 N	0	0	5 wks.
4 N	0	0	5 days

normal concentration was found to be the most satisfactory for freezing. The data of table 5 may be considerably varied by changing manufacturing conditions and temperatures. High or long heat treatment shortens the storage period in which the milk is free from casein precipitation. High storage temperatures produce the same effect.

The development of viscosity in concentrated frozen milk during storage at two different temperatures is shown by the data plotted in Fig. 2.

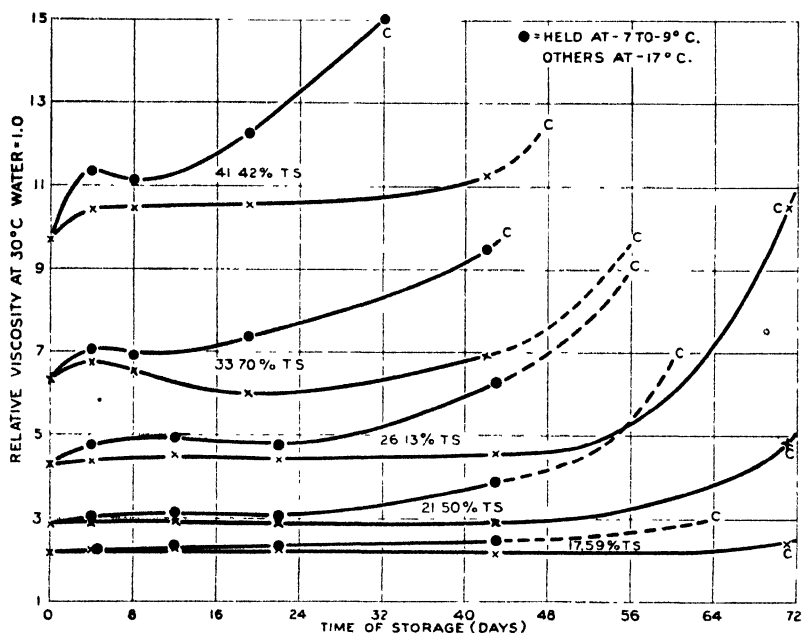


FIG. 2. THE EFFECT OF TIME OF STORAGE IN THE FROZEN STATE UPON THE RELATIVE VISCOSITY OF CONDENSED MILKS OF DIFFERENT CONCENTRATION. "C" AT THE END OF EACH CURVE INDICATES THE TIME WHEN CASEIN COAGULATION WAS FIRST FOUND.

Whole milks condensed to different percentages of solids were used. In most cases when the slight coagulation which precedes gel formation set in, viscosity measurements could no longer be accurately made. In these cases the probable course of the curves is indicated by dotted lines. The importance of low temperatures of storage in preventing the development of high viscosities is apparent.

The ability of the homogenization process to increase protein adsorption and fat clumping in cream was utilized in a method developed to separate some of the milk constituents. It was observed that when homogenized frozen cream was thawed at a temperature below the melting point of the fat, the serum could be drained from the thawing mass, leaving behind a mixture of fat and adsorbed casein. If the melting temperature was high enough to liquefy the fat the material melted as cream and no separation was possible. The fat-casein mixture could be washed with ice water without loss to remove traces of serum.

Data are given in table 6 which are representative of the composition of the fat-casein mixture obtained from creams of 10, 20 and 30 per cent

TABLE 6

*Composition of the fat-casein mixture secured from frozen and thawed cream. (Freezing time 10 to 12 hours in 1 gal. cans at  $-18^{\circ}$  C.)*

FAT IN ORIGINAL CREAM	WATER	FAT (BABCOCK)	PROTEIN (TOTAL $N \times 6.38$ )	LACTOSE AND ASH (BY DIFFERENCE)	CLARITY OF SERUM REMOVED
%	%	%	%	%	
10	53.61	35.0	7.12	4.27	very turbid
20	36.60	55.5	6.59	1.31	turbid
30	20.51	74.0	5.29	.20	clear

fat. A clear separation of serum was obtained only with creams above 25 per cent fat. When the fat content of a cream was lower than this figure there was insufficient fat present to adsorb and hold all the casein. Under such conditions some of the casein escaped with the serum.

The fat-casein mixture was found to be of some practical value. If water was added to replace the serum and the mixture warmed, much of the fat separated by oiling off from the casein. A cream separator removed all but about 0.5 to 1.0 per cent fat. The resulting casein dispersion appeared to possess all of its original characteristics. It was almost tasteless in the absence of the serum and developed very little cooked flavor or brown color after heating to sterilization temperature. Its heat stability remained about the same as that of normal milk. The product provided a normally dispersed casein which should be of value in studies of this protein in its native state. It has been used to advantage in heat stability studies in these laboratories.

The fat-casein mixture was used to raise the milk-protein solids of ice cream mixes without also increasing their lactose content. When the fat for the mix was obtained entirely from frozen cream, the procedure for using only the fat and casein of the frozen cream was very simple. The homogenized frozen cream was thawed upon a fine wire netting suspended over a receiver for the serum, the operation being conducted in an ice box held at  $5^{\circ}$  to  $15^{\circ}$  C. ( $41^{\circ}$  to  $59^{\circ}$  F.). After thawing about 24 hours the fat-casein mixture was added to the mix before pasteurization. For small quantities the most rapid method of removing the serum from the thawed cream was by means of a Büchner funnel using suction. Through use of the fat-casein mixture the protein solids of the mix were increased as much as 1.5 per cent in this manner without increasing the lactose or salts.

Low temperature thawing of homogenized frozen cream provided a simple means of obtaining milk serum in large quantities. By careful handling, a serum equal in clarity to that obtainable by ultra-filtration methods was secured. When the serum was obtained as a by-product in the preparation of the fat-casein mixture for ice cream, it was used to advantage in milk sherbet, giving it a distinctive and pleasing milk flavor.

#### SUMMARY

1. Slow freezing of milk or cream caused a gradual precipitation of the caseinate system and an immediate destruction of the fat emulsion.

2. Freezing did not alter the heat stability of skim milk until the product had been held frozen for several months at  $-18^{\circ}$  C. ( $0.4^{\circ}$  F) or below. Freezing caused an immediate increase in the amount of casein which could be centrifuged from milks heated before freezing. Freezing, therefore, caused a slow and gradual increase in the size of the casein aggregates but the change was not noticeable until the freezing period was well advanced.

3. The destruction of the fat emulsion in cream during slow freezing was lessened by adding cane sugar or increasing the solids-not-fat content of the cream before freezing. Homogenization slightly retarded fat separation when low fat creams were frozen. Freezing destroyed the fat clumps formed in cream by homogenization and restored to the cream the heat stability which it possessed before processing.

4. Fresh whole milk was pasteurized, condensed to  $\frac{1}{3}$  its weight, canned and frozen without any detrimental effects to the body or flavor of the product. This milk when held frozen at a low temperature and reconstituted at any time within a four-week period by the addition of cold water, yielded a product which often could not be distinguished from fresh market milk. Its use where fresh market milk is expensive or not available was suggested.

5. A process for the preparation of large quantities of normal undenatured casein and of milk serum was developed. Frozen homogenized cream was thawed at a temperature below the melting point of the fat; clear milk serum was collected from the melting mass and the residual mixture of fat and casein was utilized in the preparation of normal casein or to raise the protein solids of ice cream mix.

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# THE HEAT STABILITY OF EVAPORATED MILK MADE FROM HARD-CURD MILK, SOFT-CURD MILK AND MILK FROM MASTITIS INFECTED UDDERS\*

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In the course of some preliminary studies with soft-curd milk, it was found that the milk samples of low curd tension, after condensing, consistently coagulated at lower temperatures or coagulated sooner at the same temperature than did milk of high curd tension. The soft-curd product was furthermore affected erratically by added salts such as calcium acetate, sodium citrate and sodium carbonate whereas the hard-curd milk was invariably further stabilized by the citrate and carbonate and destabilized by the acetate. These results were at first interpreted as indicating some inherent qualitative difference between soft-curd milk and hard-curd milk but this conclusion was soon abandoned since exhaustive study failed to reveal such differences between the two types of milk (1). It was found, however, that udder infections lower the curd tension of some milk sufficiently to bring it under a soft-curd classification (2). In checking on the samples of milk used for the comparisons mentioned above, it was discovered that the soft-curd milk came from a group of cows, several of which reacted positively to mastitis in one or more udder quarters. It was therefore decided to repeat the comparisons using hard-curd and soft-curd milk from animals free from suspicion of udder infections and milk from animals showing sub-clinical indications of mastitis in one or more quarters as determined by the cell count, the thybromol test, and the percentage of chlorides.

## EXPERIMENTAL

### *Methods*

The milk used in these experiments came from selected cows of the college herd. These animals were chosen after several months' observation and were known to produce milk of the desired type. Each lot of milk used for comparison was composited from the complete milkings of from 5 to 10 cows, the individuals contributing to each lot being changed with each trial, although several were included in more than one trial in a different grouping. Considerable care was exercised in ascertaining that each lot of milk was true to the type desired for comparison.

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Each lot of milk was first separated, the resulting skim milk, only, being used in the experiments. This was forewarmed at various temperatures and concentrated (about 2 to 1) in a small vacuum pan at an evaporating temperature of about 135° F., cooled at once and standardized with distilled water to a milk solids content equivalent to that of the other lots of milk in the trial. Samples were sealed in six ounce tins and sterilized in a pilot sterilizer at a temperature of 240° F. Several trial sterilizer runs were made with each lot of milk until a holding interval was found which produced coagulation in at least one of the three types of milk being compared. Following this, two series of sealed samples were prepared from each of the lots of milk, one series containing increasing increments of M/4 sodium carbonate and the other series, increasing increments of M/4 calcium acetate. These were subjected to sterilization at 240° F. for the predetermined time interval and the relative degree of coagulation noted. In most cases additional sterilizing trials, using different holding intervals, were required with one of the lots of milk, and often with two lots, in order to distinguish accurately the effect of the added salt solutions on the stability. The diluting effect of the salt additions was compensated by additions of distilled water, where necessary, so that the total dilution was the same in all cases.

The "coming up" intervals in the sterilizing trials varied from 8 to 12 minutes but were held constant for every direct comparison of the types of milk.

The procedure outlined was followed in every trial and for each different forewarming temperature where these were varied.

The curd tension determinations were made according to the Hill technique (3) using Monier and Sommer's modification (4) and alcohol numbers were obtained by the method of Doan and Minster (5).

Not all of the data obtained in these experiments are presented due to the inability to condense and summarize them into convenient tables. Typical data are shown in sufficient amount to substantiate the conclusions reached.

#### *Heat Stability without Added Salts*

In every trial the condensed milk from animals afflicted with sub-clinical mastitis (hereafter termed mastitis milk) was found to be less heat stable than either the hard-curd condensed milk or the soft-curd condensed milk. This held true regardless of the forewarming temperature used. The results of eight typical trials are shown in Table 1. The data do not indicate any appreciable difference in the coagulability of hard-curd milk and soft-curd milk and from the further observations made, it is believed there is none. In one trial shown, an apparent difference is evident, sample 16S coagulating to a great degree than 16H. This however

TABLE 1

*The Relative Heat Stability of Soft-Curd Milk, Hard-Curd Milk and Milk from Udders Infected with Sub Clinical Mastitis, After Condensation*

TRIAL NO. AND SAMPLE*	CURD TENSION	ALCOHOL NO.	FORE WARM ING TEM- PERATURE	TOTAL SOLIDS CONTENT	"COMING UP" TIME	TIME HELD AT 240°F	DEGREE OF COAGU- LATION*
	GRAMS	mls	°F	min	min		
6	M	34	170	16.81	8	17	4
	H	62	170	16.86	8	17	0
8	M	29	170	17.90	8	15	1
	H	64	170	17.78	8	15	0
13	M	58	8.8	205	11	15	3
	H	76	8.5	205	11	40	0
	S	35	9.2	205	11	40	0
14	M1	41	8.4	205	11	15	1
	M2	41	8.4	205	11	23	4
	H	70	8.5	205	11	23	0
	S	32	9.5	205	11	23	0
15	M	46	8.8	205	11	15	2
	H	74	8.2	205	11	42	0
	S	34	8.8	205	11	42	0
16	M	47	8.6	180	12	30	2
	H	66	8.4	180	12	40	0
	S	34	9.2	180	12	40	2
17	M1	49	8.4	205	12	30	2
	M2	49	8.4	180	12	30	0
	H1	63	8.8	205	12	40	1
	H2	63	8.8	180	12	40	0
	S1	32	9.4	205	12	40	0
	S2	32	9.4	180	12	40	2
18	M	53	200	17.67	9½	15	1
	H	105	200	17.81	9½	15	0

\* M - Milk from udders affected with sub-clinical mastitis.

H - Milk from normal hard-curd milk.

S - Milk from normal soft-curd milk.

+ 0 = no visible coagulation; 1 = slight coagulation; 2 = moderate coagulation;

3 = prominent coagulation; 4 = pronounced coagulation; 5 = complete coagulation.

was the only comparison showing a definite variation. Some differences are evident in trial 17 but these are partly due to the rather considerable variation in the milk solids content of the samples.

The stability of the soft-curd milk in the fluid state toward alcohol

was greater than either the hard-curd milk or the mastitis milk. This is probably a reflection of the lower casein content of the soft-curd milk. The mastitis milk did not show a consistent difference from the hard-curd milk in this respect.

The data obtained for trial 17 indicate that the high temperature of forewarming (205° F.) stabilized mastitis milk to a lesser degree than did the low temperature (180° F.) This is contrary to the usual effect of varying forewarming temperatures on normal milk. While the data presented are not sufficient to be conclusive on this point other results obtained in the study were confirmatory and the data dealing with the effect of added salts (Table 2) also support the opinion.

#### *Heat Stability with Added Salts*

When increasing quantities of sodium carbonate and calcium acetate were added to the condensed milk samples, prepared as previously described, the stability of the hard-curd milk and soft-curd milk was affected for the most part in a normal manner. The carbonate usually increased the resistance toward coagulation while the acetate decreased it. The data presented do not show this effect as well as might be wished but other data and examination of the samples for viscosity indicate it to the satisfaction of the authors. The mastitis milk, however, when forewarmed at the higher temperatures, in the majority of cases reacted oppositely, being destabilized by additions of the carbonate and stabilized by additions of the acetate. At the lower temperatures of forewarming the results with mastitis milk were not so consistent. In the majority of cases stabilization or partial stabilization was obtained with the carbonate and destabilization with the acetate. In frequent cases, however, the results were directly reversed and similar to the effects when the forewarming temperature was high. The transition point appeared to be in the neighborhood of 180° F. forewarming temperature. The results obtained, with the same trials shown in Table 1, are presented in Table 2.

While a few discrepancies in some of the data make direct comparisons uncertain, the results are sufficiently uniform to indicate that mastitis milk has a different so-called "salt-balance" after forewarming and condensing than does normal milk. Apparently it is less stable regardless of forewarming treatment than normal milk and exhibits a decided tendency to be stabilized by calcium when forewarmed at temperatures commonly used for evaporated milk.

#### *Effect of Mixing Mastitis and Normal Milk*

Since mastitis milk and normal milk appeared to have ionic maladjustments which might be expected to compensate each other if mixed, some mixtures were compared (in the manner described previously) with normal

TABLE 2

*The Effect of Sodium Carbonate and Calcium Acetate Additions on the Relative Heat Stability of Soft-Curd, Hard Curd and Mastitis Milk after Condensation*

TRIAL NO AND SAMPLE		FORE- WARM- ING TEM- PERA- TURE °F	DEGREE OF COAGULATION* OBTAINED IN STERILIZING WHEN INDICATED AMOUNTS OF SALTS WERE ADDED TO A 6 OUNCE TIN									
			Con- trol	M/4 Na <sub>2</sub> CO <sub>3</sub>				M/1 Ca (C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) <sub>2</sub>				
				0 0 0 0				0 2 0 4 0 6 0 8				ml. Reagent
				0 0	0 2	0 4	0 6	0 8	0 2	0 4	0 6	0 8
6	M	170	4	3	3	3	1	4	4	5	5	
	H	170	0	0	0	0	0	0	0	1	2	
8	M	170	1	0	0	0	0	1	2	3	5	
	H	170	0	0	0	0	0	0	0	0	0	
13	M	205	3	3	4	4	5	2	1	0	0	18.09% Milk Solids
	H	205	0	0	0	0	0	0	0	0	0	18.09% Milk Solids
	S	205	0	0	0	0	0	0	0	0	0	19.02% Milk Solids
14	M1	205	1	2	2	3	3	1	0	0	0	Held 15 Min. @ 240°F.
	M2	205	4	5	5	5	5	4	3	3	2	Held 23 Min. @ 240°F.
	H	205	0	0	0	0	0	0	0	0	0	Held 23 Min. @ 240°F.
	S	205	0	0	0	0	0	0	0	0	0	Held 23 Min. @ 240°F.
15	M	205	2	3	3		4	2	1	0	0	Held 15 Min. @ 240°F.
	H	205	0	0	0	2	4	0	0	0	1	Held 42 Min. @ 240°F.
	S	205	0	0	0	0	1	0	0	0	0	Held 42 Min. @ 240°F.
16	M x	180	2		3	4	4	1	0	0	0	Held 30 Min. @ 240°F.
	H	180	0	0	0	0	0	0	0	0	1	Held 30 Min. @ 240°F.
	S	180	2	2	0	0	0	2	2	3	3	Held 30 Min. @ 240°F.
17	M1	205	2	1	3	4	5	2	1	0	0	Held 40 Min. @ 240°F.
	M2	180	0	0	0	1	2	0	0	0	1	Held 40 Min. @ 240°F.
	H1 z	205	1	1	2	3	4	0	0	0	0	Held 40 Min. @ 240°F.
	H2	180	0	0	0	1	1	0	0	0	1	Held 40 Min. @ 240°F.
	S1	205	0		0	0	0	0		0	1	Held 40 Min. @ 240°F.
	S2	180	2	1	0	0	0		3	4	5	Held 40 Min. @ 240°F.
18	M	200	1	1	2	2	2	0	0	0	0	
	H	200	0	0	0	0	0	0	1	2	2	

\*M - Milk from udders affected with sub-clinical mastitis.

H - Milk from normal hard-curd milk.

S - Milk from normal soft-curd milk.

+ 0 = no visible coagulation; 1 = slight coagulation; 2 = moderate coagulation;  
3 = prominent coagulation; 4 = pronounced coagulation; 5 = complete coagulation.

x - Lower milk solids content than other samples.

z - Higher milk solids content than other samples.

milk with respect to heat stability. The data are so heterogeneous that they cannot be arranged satisfactorily in tabular form and are therefore omitted. The results, however, indicate that the presence of mastitis milk in normal milk invariably destabilizes the normal milk. Considering the situation from the opposite standpoint, normal milk added to mastitis milk improves the stability of the latter and also changes it in such a way that calcium additions no longer stabilize it. It should be appreciated that these trials were in the nature of preliminary studies and further comparisons, more carefully controlled, will be necessary before the effects of mixing mastitis and normal milk on heat stability can be completely appreciated.

#### DISCUSSION

In attempting to understand or explain the differences in the heat stability of mastitis milk and normal milk, the mineral or salt composition of milk from diseased udders becomes of consequence. Data on this subject are rather meagre but Hashimoto (6), Schrodtt (7), Sommer (8) and Rosell (9) have presented figures which can be taken as typical. These are shown in Table 3, together with average values for normal milk. Rosell's data are averages for infected and clean quarters of the same animals and therefore illustrate very accurately the changes due to mastitis.

TABLE 3  
*Percentage Composition of the Ash of Normal and Mastitis Milk*

INVESTIGATOR	K <sub>2</sub> O	Ca O	Na <sub>2</sub> O	Mg O	P <sub>2</sub> O <sub>5</sub>	Cl
Mastitis Milk						
Hashimoto	8.98	7.44	36.54	1.74	17.38	33.63
Schrodtt	10.56	16.17	24.92	2.70	24.56	24.52
Sommer	5.08	7.52	42.37	2.88	8.76	44.64
Rosell (Recalculated)	17.85	16.86	35.73	2.08	16.41	34.12
Normal Milk						
Sommer	24.65	23.42	8.18	2.79	26.28	13.95
Rosell (Recalculated)	19.13	29.45	15.41	2.48	32.76	15.93

Udder infections unquestionably decrease the calcium, potassium and phosphorus content of milk and increase, very markedly, the sodium and chlorine. In view of the rather delicate balance between the various ions of milk necessary for maximum stability of the casein toward heat (10) (11) (12), such changes in the mineral constituents would be expected to influence the heat coagulation point markedly. One effect, apparently, is to render the mastitis milk decidedly more heat sensitive than normal milk after concentration to a solids content comparable to that of evaporated milk. The mastitis milk seems to be unbalanced in the direction of too little calcium. This is indicated by the stabilizing effect of added

calcium acetate and also by the tendency of high forewarming temperatures to destabilize the milk more than low temperatures.

Milk from mastitis udders is low in hydrogen-ion concentration. This in itself may be an important factor in the poor stability of such milk, since Benton and Albery (11) have shown that the pH effect may overshadow the effect of added ions outside the pH ranges of 6.58 to 6.65. It may be argued therefore that the beneficial effects of adding calcium acetate to mastitis milk are a result of a lowering of the pH rather than any direct effect of the cation. It is impossible from the present data to determine which of these probabilities is the important one.

The considerable increase in the sodium chloride content of mastitis milk would increase the osmotic pressure were it not for the fact that a corresponding decrease in lactose occurs, which maintains the milk isotonic with blood (14). Nevertheless the mastitis milk contains a higher concentration of ionized electrolytes as indicated by the increased conductivity (14). The presence of this excess of electrically active particles might be expected to destabilize milk casein toward heat or any other agency of coagulation.

Another explanation of the reduced heat stability of mastitis milk might also be offered, based on the increase in the albumin content characteristic of such milk. Sommer (15) has shown experimentally that increasing the albumin content of milk lowers its resistance to coagulation in the condensed form. Since the increase in albumin content of mastitis milk, however, takes place at the apparent expense of casein (16), it seems unlikely that the increase in albumin would more than compensate the decrease in casein.

The literature dealing with the heat stability of milk lacks any definite statements regarding the presence or absence of milk from cows affected with mastitis. Apparently this factor has been neglected in most, if not all, of the studies made on the subject. It is believed that the peculiarities exhibited by some of the samples of milk used by the various workers might have been related to the presence of mastitis milk. Several of the investigators (10) (11) (12) (13) have indicated that there are two general types of milk (omitting the effects of bacterial acidity and enzymes). The most common type is that which is stabilized by high forewarming temperatures and additions of such anions as di phosphate, carbonate, citrate, borate, etc. The less common type, limited for the most part to small lots of milk or milk from individual cows, is that stabilized by additions of acid and such cations as calcium or magnesium. This latter type of milk is suspiciously similar in stability characteristics to the mastitis milk utilized in this study. Because of the wide-spread occurrence of mastitis of the sub-clinical type, it is reasonable to suppose that some of the work that has been carried out on the subject of heat coagulation has been complicated by this apparently disregarded factor.

## CONCLUSIONS

There seems to be no appreciable difference between concentrated soft-curd milk and concentrated hard-curd milk with respect to sensitivity toward heat coagulation and with respect to the stabilizing or destabilizing effects of forewarming and the addition of certain anions and cations.

Concentrated milk from animals infected in one or more quarters with sub-clinical mastitis is decidedly less stable toward heat than normal milk. Such milk seems to react oppositely to most normal milk in that it is further destabilized at the higher forewarming temperatures and by additions of sodium carbonate and is further stabilized by additions of calcium acetate. In these particulars, at least, it is very similar to one of the two "types" of milk noted by previous investigators, namely, that type, only occasionally encountered, which is stabilized by bacterial action, mixing with it "poor quality milk," and by additions of acids, and cations such as calcium and magnesium.

Mixing mastitis milk with normal milk tends to destabilize the latter.

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## FIVE YEARS RESULTS ON MONTHLY CLIPPING OF PASTURES

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Eleven or more caged pasture areas at Baton Rouge have been clipped at monthly intervals for the growing seasons of 1930-34. Four of these areas were in seeded bench land pasture, while the others were in recently broken alluvial land. The soil is nearly neutral in reaction and high in fertility. The prevailing vegetation in early spring was White Dutch clover (*Trifolium repens*) except on bench land, where much hop clover (principally *Trifolium procumbens*) occurred. Rye grass (*Lolium* sp.), Canary grass (*Phalaris* sp.), and common oats were present in varying amounts in different seasons. This early vegetation was gradually replaced in May by Dallis (*Paspalum dilatatum*) and Bermuda (*Cynodon dactylon*) grass, with a small amount of carpet grass (*Axonopus compressus*) during wet summer months. White clover reappeared during September and October with favorable growing conditions. Some minor grasses and edible weeds occurred at times. The term "grass" is used hereafter for convenience to include all of the above edible vegetation. Inedible vegetation so far as discernible was plucked out of each area just before cutting.

Eight of the areas used were 3 × 7 feet and the rest 2 × 2 feet in size. With the exception of two areas in oats, or cage destruction by cattle, the same location was used continuously for five years. These caged areas were closely cut with a grass hook the first week in March of each year, and at approximately 30-day intervals thereafter during the growing season. The intervals were sometimes extended to avoid clipping grass on a rainy day or holiday. Because of slow growth, June and early July were combined in one cutting. The entire weight of fresh grass was recorded and individual samples taken for analysis. The average height of grass was determined by three measurements prior to clipping. It was observed during the first two years that samples of the same kind of grass cut on the same date checked closely, and thereafter one composite sample was used for similar grass each date. Analysis were by standard methods adopted by the Association of Official Agricultural Chemists.\* A 400 gram portion of each sample of grass was dried to constant weight at room temperature and from this the hay yield determined. The nutritive value (T. D. N.) was calculated from the coefficient of digestibility of peas and oats for early cuttings and for those of Bermuda for grass cut after May.

\* The aid of A. P. Kerr, Chief Chemist, and associates, is acknowledged in making all chemical analyses.

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TABLE 1  
*Composition of pasture grasses cut monthly 1930-1934, Baton Rouge, La.*

DATE OF GROWTH	RAIN FALL INCHES	NO. SAMPLES	HEIGHT INCHES	GRASS PER ACRE POUNDS	DRY MATTER PER ACRE LBS.	DRY MATTER IN 100 POUNDS GRASS (LBS.)	PER CENT OF DRY MATTER					T. D. N. PER 100 GRASS (EST.) LBS.
							Crude protein	Fat	N. P. E.	Fiber	Ash	
February	5.79	47	4.1	4,302	909	21.13	21.34	4.06	42.35	21.89	10.36	13.31
March	4.00	57	5.8	6,149	1,117	18.17	20.32	3.71	44.03	21.94	10.00	11.72
April	5.02	60	7.2	8,078	1,627	20.14	18.81	3.56	42.05	25.18	10.40	12.65
May	5.38	56	4.6	4,446	1,101	24.76	16.95	3.38	41.69	26.84	11.14	15.17
June } July }	4.27	58	5.9	4,637	1,370	29.54	13.49	2.66	46.47	27.63	9.75	14.97
August	5.96	61	8.2	7,248	1,735	23.94	12.56	3.11	44.26	29.89	10.18	12.02
September	5.12	57	7.1	5,608	1,404	25.03	11.64	3.32	45.02	29.96	10.06	12.36
October	5.35	55	4.9	3,539	1,015	28.67	12.43	3.14	44.58	29.27	10.58	14.05
Season												
1930	34.52	68		39,566	8,386	21.70	15.21	3.03	42.94	27.12	11.70	13.20
1931	32.10	95	39.5	49,979	12,330	24.67	16.81	3.45	44.97	25.31	9.46	13.38
1932	48.03	96	43.9	39,660	10,050	25.34	15.60	3.35	44.76	26.58	9.95	13.93
1933	40.00	104	52.3	41,143	9,570	23.26	15.19	2.81	44.97	27.40	9.63	12.41
1934	49.48	88	51.7	35,967	7,963	22.14	14.73	3.39	42.67	28.78	10.43	13.09
Ave. 5 yrs.	39.82	451	46.8	41,263	9,667	23.43	15.51	3.21	44.06	27.03	10.19	13.20

Table 1 presents seasonal and yearly weighted averages of these grass clippings. The date of growth is for the larger part of the month listed. "February grass" was actually cut the first week in March. Likewise, the rainfall is for the period prior to clipping, rather than for the calendar month. Because of a late season and delay in placing cages, no samples were obtained early in 1930. We have been impressed all through this work by three important points, two of which are evident in glancing at this table. First, that the grass yields reached two peaks, one for April and the other in August. The first is explained by the heavy yield of clover under the first favorable growing conditions of spring. This growth in turn so shades Bermuda and other summer grasses that several weeks of slow growth occur after the clover disappears. Distribution of rainfall is also poor as compared to late summer. Cooler night temperatures and lack of root reserve slows growth of grass in September and October. In grazing practice, one or more fields are allowed to grow for hay in May, all grazed in June and early July, and again surplus pastures cut for hay in August or September. That necessitates two or more fenced pastures for one dairy herd. Second, the early spring grass contains nearly twice as much crude protein as that cut in September. A gradual decline in protein and moisture content occurs with advancing season while fiber content increases. Fat content apparently decreases slightly with advance in season, but nitrogen free extract and total ash were quite constant. Because of these changes, the computed total digestible nutrients per 100 pounds of grass tend to rise to 15 in mid-summer and then gradually decline. If the following coefficients of digestibility suggested by the pasture committee (1) (crude protein 75; crude fiber 79; nitrogen free extract 80; and fat 50) are used for all samples, the nutritive value of all clippings average about 27 per cent higher. The difference is even greater for late summer. One hundred pounds of September grass would contain 18.06 pounds, or 46.2 per cent more total digestible nutrients by the use of these coefficients than computed in Table 1. The true value is probably somewhat less than these latter figures. As pointed out in a previous article (2), changes in protein are not as abrupt as the results indicate. The high percentage of non-protein nitrogen in immature grass gives a higher crude protein figure than actually exists. Late summer clippings are undoubtedly more mature, throughout representing the same length of growing time. Feeding practices may be advantageously changed to fit pasture yields and composition, however. A third fact brought out has been the uniformity in analysis of grass samples clipped the same date, regardless of species, genera, or even family. Italian rye grass and oats cut in early spring are very similar in chemical analysis to clovers grown during the same period. Pure samples of Dallis, Bermuda, or carpet grass cut on the same date were much alike

in analysis. Rate of growth as affected by distribution of rainfall or temperature evidently is more important than species of plant in the resulting effect on chemical composition.

Total rainfall for the period or during preceding period had a rather indirect effect on grass growth. The least rainfall occurred in 1931, the year of greatest growth, while the heaviest rainfall occurred in 1934, the year of lowest yield. Even distribution of rainfall, especially during the growing months of May, June, July, was responsible for large yields in 1931, and the poor distribution for lower yields in 1930 and 1934. In general, low average minimum and maximum temperatures within a period were recorded when the best yields were obtained. This was especially true during the spring months of clover growth.

During the first two years, nitrate and superphosphate were applied to duplicate areas. Neither had any effect on protein content of the grass. This checks with results obtained by the Florida (3), South Carolina (4), Delaware (5) stations, and other Louisiana work (6). Fertility causes faster and more total growth rather than affecting the plant's chemical composition.

Sixty composite samples, collected during the first four years and air-dried, averaged 0.989 per cent calcium oxide and 0.778 per cent phosphorus pentoxide as previously reported (7). Late summer clippings averaged much lower in calcium and slightly lower in phosphorus than spring growth. No samples were low enough to indicate that an animal might suffer from a lack of these minerals if receiving all necessary nutrients from the grass.

One observation for which we have as yet no explanation is that where cages were not moved in five years, the amount of clover appears to be less and grasses have increased in the early spring.

#### SUMMARY

1. Pasture yields were higher in April and August than other months when clipped at 30-day intervals for five years.

2. February grass, calculated on a dry matter basis, contained nearly twice as much crude protein as that cut in September. Calcium, and to some extent phosphorus, decreased from spring to late summer. Dry matter and fiber content increased as the season advanced, while other constituents were quite uniform.

3. Samples of grass representing the same growth period were quite similar in chemical analyses regardless of species or genera or fertilizer.

4. Distribution of rainfall, maximum and minimum temperatures, and shading are some other factors that influence rate of growth and consequent analysis and yield of pasture vegetation under the condition of this experiment.

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## OXIDIZED FLAVOR IN MILK

### I. THE PROBABLE RELATION OF LECITHIN TO OXIDIZED FLAVOR\*

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It is generally believed that oxidized flavor is the taste-result of the oxidation of butterfat in milk. This belief has arisen from the fact that the flavor, once developed, tends to remain with the butterfat portion of milk during processing; and that the flavor never has been known to develop in skimmilk. However, one exception has been observed in the case of buttermilk, in which oxidized flavor has been known to occur (5).

In 1916 Guthrie (2) reported that his results "seemed to indicate that metallic flavor developed more rapidly and to a greater extent in cream that was rich in butterfat than in cream with a low fat content. The flavor was very strong in samples of soft-cream cheese, but was never noticed in cottage cheese which contains practically no butterfat. Whenever the flavor was found in whole milk it was always near the surface, in the cream, and it was never observed in skimmed milk. For some reason, however, it was often found in buttermilk. With the exception of buttermilk, metallic flavor was never found in a dairy product that was low in butterfat content." It is questionable whether the metallic flavor referred to by Guthrie is identical with so-called oxidized flavor. The term oxidized flavor, referring to the off-flavor caused in milk by dissolved copper or iron, sometimes has been called cappy, cardboard, metallic, oily, tallowy, oily-tallowy, emery, and corundum. Tracy, Ramsey, and Ruehe (6) have shown that small amounts of copper dissolved in milk fail to produce tallowy (oxidized) flavor when considerable growth of microorganisms has occurred, yet the metallic flavor observed by Guthrie developed in cream and buttermilk during and after the growth of lactic acid bacteria in amounts sufficient to cause souring. Nevertheless Guthrie's observations of the development of metallic flavor in sour buttermilk are strikingly similar to those of the authors in the case of the development of oxidized flavor in sweet-cream buttermilk.

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<sup>2</sup> Department of Agricultural Chemistry. Most of the data presented herein were included in a thesis presented by W. Carson Brown in partial fulfillment of the requirements for the degree of Master of Science, West Virginia University.

The development of oxidized flavor in buttermilk, when no other dairy product of like butterfat content supports this flavor, suggests that the lecithin adsorbed on the fat globules is the constituent first affected when oxidation occurs, and that the butterfat may be oxidized little or not at all in the average case. The finding (5) that lecithin purified from dry buttermilk and having become oxidized during purification imparts a typical oxidized flavor to skimmilk when dispersed in it lends further support to this suggestion. In addition, the report of Henderson and Roadhouse (3) that milk fat from milk of oxidized flavor has passed only a fraction of its oxygen-uptake induction period indicates little likelihood that the oxidation of the milk fat had proceeded far enough to become oxidized or to impart oxidized flavor. The facts that at least one double bond occurs in the milk-lecithin molecule; that lecithin in purified state oxidizes readily; and that lecithin makes up a portion of the adsorbed layer on the butterfat globules and, therefore, probably is exposed to the action of oxidizing agents before the butterfat, are further evidence in support of a belief that lecithin, rather than butterfat, is the constituent affected when oxidized flavor develops.

#### EXPERIMENTAL

##### *Removal of Adsorbed Substances*

If the constituent in which oxidized flavor develops is one of those adsorbed on the butterfat globules in milk it should be possible to obtain butterfat free of oxidized flavor by removal from the globules of adsorbed materials. Accordingly, six trials designed to accomplish this objective were planned. Five of these trials were carried out in the manner described below.

Twenty gallons of selected milk, in well-tinned ten-gallon cans, were placed in cold storage immediately after milking. Morning's milk was used in each case, and pasteurization followed milking within two hours. Pasteurization was accomplished by placing the cans of milk in a tank fitted with a water inlet and a steam jet, heating to 144° F. and holding for 30 minutes. Into one can of this milk was placed 0.01 per cent of powdered copper before pasteurization, whereas the other milk was not treated but was used as a control batch. After pasteurization both batches were cooled. The milk to which no copper had been added was cooled to about 70° F. and processed immediately by a procedure which will be described later. The milk to which powdered copper had been added was cooled to 40° F. and placed in two large glass bottles which then were immersed in ice water, and air was bubbled through the milk for 24 hours. This treatment was found to cause the development of a strong oxidized flavor. Previous experiments had shown that no off-flavors were produced by air treatment of milk to which no copper had been added. The milk

then was heated to 65 or 70° F. and separated. The cream was standardized to 35 per cent butterfat with skimmilk just separated from it.

The cream from each batch was divided into two lots. Lot 1 cream was churned in a glass churn and its butter and buttermilk tasted to detect the presence of any oxidized flavor.

Lot 2 cream was converted into washed butterfat in order to remove from the butterfat the substances adsorbed on the fat globules. The cream was diluted to a volume of about three gallons with water at approximately 100° F., separated by means of a De Laval No. 17 separator, rediluted, and separated until the cream had been washed 14 times or more. Near the end of this procedure a noticeable oiling off of the butterfat occurred, which indicated that the fat globules no longer were stabilized. Further washing caused a complete oiling off of the butterfat.

In order to determine whether the treatment described above caused the removal of the lecithin contained in the film originally adsorbed on the fat globules, three different butterfats prepared in this way were analyzed for organic phosphorus. For this determination the fats were fused in a mixture of two parts sodium carbonate and one part potassium nitrate. After fusion the mixture was dissolved in hot water and hydrochloric acid and phosphorus was determined by the colorimetric method of Fiske and Subbarow (1). No trace of phosphorus was found in any of the samples, thus indicating that the washing removed all or practically all of the phospholipids adsorbed on the fat globules in the original milk.

The washed butterfat was redispersed in fresh skimmilk of good flavor by homogenizing at a pressure such that the fat globules were approximately of the same size as those in the original whole milk as shown by comparison under the microscope. Milk prepared in this way will be referred to as remade milk.

An attempt was made to develop oxidized flavor in the remade milks in order to determine whether or not development is possible in milk containing butterfat globules from which the "hull" substance has been removed. Accordingly such remade milk was treated with 0.01 per cent of copper powder, pasteurized and air bubbled through as described above except that the air treatment was continued for 36 hours.

To determine if the butter oils from these batches were oxidized, a Kreis test was made on each fat. The results did not indicate even the slightest degree of oxidation in any of the samples of butter oil.

The control batch of milk differed from the experimental batch only in that no copper was added to it and no air treatment given. It was separated fractioned in the same manner as the experimental batch.

A sixth experiment was conducted in the same manner as the five described above except that the oxidized flavor was developed by the method of Tracey, Ramsey and Ruehe (6). Their method consisted of adding a

soluble copper salt to the milk at the rate of 2.6 parts per million of copper and storing at a low temperature until the oxidized flavor developed. A storage period of 24 hours was required

The results of the six experiments described above are shown in Table 1.

TABLE 1  
*The distribution of oxidized flavor in milk fractions—(Results of six trials)*

FRACTION	*CONTROL	*TREATED WITH 0.01% CU
Original milk	—	—
After pasteurization	—	—
After air treatment	—	+++
Cream	—	++++
Skimmilk	—	+++
Butter	—	++++
Buttermilk	—	++++
Washed butterfat	—	+
Remade milk	—	—

Studies of Remade Milk

1. Pasteurized with copper	—	—
2. Pasteurized without copper	—	—
No. (1) Submitted to air treatment	—	—
No. (2) Submitted to air treatment	—	—

\* + Indicates oxidized flavor present.

— Indicates no oxidized flavor present

The results of the foregoing experiments indicate that a large proportion of the oxidized flavor occurred in the substances adsorbed on the fat globules. When these substances were removed from the butterfat and the butterfat redispersed in fresh skimmilk of good flavor, no oxidized flavor could be detected in the resulting remade milk. Of still greater significance is the fact that treatment of this milk by the procedure known to cause the development of oxidized flavor in normal milk failed to develop oxidized flavor in the remade milk

Flavor determinations on the samples of the oxidized-flavored milk showed the intensity of the flavor to be increased in cream, buttermilk, and butter, and to have about the same degree of intensity in the skimmilk as in the whole milk. An examination of the data of Holm, Wright, and Deysher (4) given in Table 2 shows that the phospholipid content of these fractions is closely correlated with the intensity of the oxidized flavor found in the fractions studied in the experiments here reported.

*Use of oxidized butterfat:* During the course of tasting samples of milk of oxidized flavor it seemed to the authors that the oxidized flavor

TABLE 2  
*\* The phospholipid content of milk and its products*

PRODUCT	FAT CONTENT	PHOSPHOLIPID CONTENT
Whole milk	2.55	.152
	3.80	.158
	4.34	.153
Skimmilk	0.07	.137
	0.11	.129
	0.10	.123
Cream	27.00	.224
	34.00	.269
	42.00	.284
Butter	86.5	.224
	85.7	.247
	85.5	.269
Buttermilk	.96	.284
	1.54	.318
	1.64	.382

\* Excerpts from the data of Holm, Wright, and Deysher (16).

was not typical of that of oxidized or slightly tallowy butterfat. Accordingly an experiment was planned to make a study of this point.

Butterfat free or practically free of lecithin was prepared by the wash-and one short tube. The flask then was placed in an oven controlled at 75° C  $\pm$  0.5°, and oxygen was bubbled through the butter oil until it became tallowy. The tallowy butter oil then was dispersed in skimmilk of good flavor by means of homogenization, the fat percentage of the remade milk being kept at about 4 per cent.

The tallowy flavor of this remade milk was pronounced and disagreeable. By successive dilutions with normal 4 per cent milk of good flavor, milks having varying intensities of tallowy flavor were obtained. When these samples were compared with those in which oxidized flavor had been produced by means of the addition of small amounts of ferrous chloride, the flavor of the samples containing the tallowy butterfat was distinctly different from that of the samples in which oxidized flavor had been produced. Even the most highly-diluted samples containing the tallowy butterfat yielded the typical tallowy flavor of oxidized butter oil, whereas the oxidized flavor of the milk samples treated with ferrous chloride was less disagreeable and more nearly resembled what may be termed oily-

stale. The latter flavor was similar to, if not identical with, the flavor previously produced in skimmilk by the dispersion in it of oxidized lecithin.

The experiment described above was repeated and the results obtained were identical. The experiment also was repeated with the variation that the butter oil was only slightly oxidized before being dispersed in skimmilk. In this case, as before, the flavor of the remade milk was tallowy, whereas the milk in which oxidized flavor had been developed by means of the addition of ferrous chloride and storage at low temperature was distinctly different in flavor, having an oily-stale taste.

#### CONCLUSION

Lecithin, rather than butterfat, appears to be the constituent of milk affected when oxidized flavor develops.

These results indicate that so-called oxidized flavor is not identical with the tallowy flavor of oxidized butterfat.

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# A STUDY OF FACTORS INFLUENCING THE SEPARATION OF WHEY IN ICE CREAM MIXES CONTAINING VEGETABLE STABILIZERS\*

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## INTRODUCTION

Various vegetable products, composed chiefly of gums, have been placed on the market as ice cream stabilizers. Because of their lower cost, these substances have been suggested for use in place of gelatine to stabilize the ice cream mix, and to aid in producing a smooth ice cream, free from objectionable ice crystals.

Studies on the efficiency of the vegetable stabilizers have been made by Prescott, Heifetz, and Stanley (1), Caulfield and Martin (2), and Lucas and Gould (3). All of these investigators noted a wheying off of the ice cream mix containing certain of the vegetable products after a few days storage. The solids of the mix showed a tendency to rise to the surface leaving a comparatively clear whey in the lower portion of the container.

Caulfield and Martin (2), assumed the wheying off of the mix to be caused by an enzyme present in the vegetable stabilizer since heating of the mix to 175° F. for 5 minutes decreased the whey separation. In another study (4), it was noted that the separation increased with an increase in the storage temperature, and decreased as the storage temperature was lowered.

## SCOPE OF INVESTIGATION

Because of the lack of information regarding the separation of whey in ice cream mixes stabilized with vegetable products, and because of its direct commercial importance, this defect was subjected to a more intensive study to determine the effects of certain practices on the extent of the whey separation.

The several factors considered in this study are: variations in the amount of stabilizer used; heating the stabilizer; different temperatures of pasteurization of the mix; heating the milk products used in the ice cream mix; varying amounts of serum solids; varying amounts of fat; and incomplete cooling of the ice cream mix.

## PROCEDURE

Only one stabilizer was selected for this study. The vegetable product used was the one causing the greatest wheying off of the ice cream mix in

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a previous study (3). This was also the stabilizer listed as "C" in the work of Caulfield & Martin (2) who found that the product contained 9.79 per cent moisture, 35.5 per cent gum, 50 per cent reducing sugar, 2.75 per cent protein, and 0.45 per cent ash. It was thought that the findings secured from a study of this one stabilizer could be applied as well to those other stabilizers which, when used in an ice cream mix, bring about whey separation to a greater or lesser degree.

An ice cream mix containing 12 per cent fat, 15 per cent sugar, and 10 per cent serum solids was used. The ingredients of the mix were 30 per cent cream, 4 per cent milk, dried skim milk testing 97 per cent serum solids, and the stabilizer. Unless otherwise specified, approximately 0.4 per cent of the stabilizer was used throughout the trials. The stabilizer was added with the sugar after the mix had been brought to a temperature of about 100° F.

Small eighteen pound batches were prepared, pasteurized at the desired temperature in three-gallon cans in a water bath, and then viscolized. The batches were cooled to about 75° F. by pouring over a tubular surface cooler through which water was running. Small samples of each batch were further cooled to 50° F. by placing them in ice water. After cooling, samples were placed in 100 cc. graduates and stored in an electric refrigerator maintaining a temperature between 42° F. and 46° F. The whey separation was observed after varying periods of storage.

In those trials in which the powdered stabilizer was heated, the heating was carried out by using the solids plate and solids vacuum oven of a Mojonniere tester. The solids plate was maintained at 180° C. and the vacuum oven at 100° C. A vacuum of 20 inches was maintained in the vacuum oven during the drying. The powder was spread uniformly over the bottom of a flat pan approximately five by nine inches in size.

When either a suspension of the stabilizer or a mixture of the milk products used in the mix was heated to high temperatures, the substance being heated was placed in a round bottom glass flask suspended in a salt solution.

## RESULTS

### *The Effect on the Whey Separation of Varying Amounts of the Stabilizer*

Although the recommended amount of the stabilizer to use was 0.4 per cent, variations by 0.1 per cent in the concentration from none to 0.5 per cent were tried, and the mix examined for whey separation after different periods of storage. The results are given in table 1.

The results showed that concentrations of the stabilizer as low as 0.1 per cent caused a separation of the whey. However, it was observed that in the samples containing 0.1 per cent, the separation was never as distinct

TABLE 1  
*The effect on whey separation when different amounts of the stabilizer were used.*  
*(Ave. 4 trials)*

PER CENT STABILIZER USED	PER CENT WHEY SEPARATION AFTER				
	4 hrs	8 hrs	24 hrs.	48 hrs	72 hrs
0.0	0.00	0.00	0.0	0.0	0.0
0.1	0.00	0.20	1.5	4.0	8.0
0.2	0.56	1.70	7.0	23.0	27.0
0.3	0.25	2.30	17.0	43.0	51.0
0.4	0.00	0.20	14.0	41.0	49.0
0.5	0.00	0.20	17.0	47.0	51.0

as in those samples with larger amounts of the stabilizer. Furthermore, the separated whey was usually extremely milky in appearance.

It is interesting to note that the whey separation appeared more rapidly in the samples containing only 0.3 to 0.4 per cent than in those containing 0.5 per cent. The appearance of whey after only four hours of storage did not occur generally throughout the trials. After eight hours of storage, however, many of the samples containing 0.3 and 0.4 per cent of the stabilizer showed slight whey separation.

After 24 hours of storage, the amount of whey separation varied directly with the amount of the stabilizer used. There was little difference between the total whey separation after 72 hours in the samples containing 0.3 per cent or more of the stabilizer. An average of about 50 per cent of whey showed at the final reading.

*The Effect on Whey Separation of Different Temperatures of  
 Pasteurization of the Mix*

Since it had previously been noted (2) that heating the ice cream mix containing the vegetable stabilizer to 175° F. for about five minutes decreased the amount of whey separation, a further study was made to ascertain the effect of different pasteurizing temperatures on the appearance of whey.

The ice cream mix was divided into three lots. Lot 1 was pasteurized at 145° F. for 30 minutes, Lot 2 was pasteurized at 165° F. for 30 minutes,

TABLE 2  
*The effect on whey separation when the mix was pasteurized at different temperatures.*  
*(Ave. 6 trials)*

LOT NO.	TEMP. OF PAST (°F.)	PER CENT WHEY SEPARATION AFTER			
		24 hrs	48 hrs	72 hrs	96 hrs
1	145	4.0	23.0	38.00	45.0
2	165	0.3	5.0	17.00	26.0
3	175	0.0	0.2	1.4	4.7

and Lot 3 was pasteurized at 175° for 30 minutes. The amount of whey separation noted in these samples after storage is given in table 2.

These averages show distinctly the beneficial influence of higher pasteurizing temperatures on eliminating, to a large extent, the separation of whey. Apparently, there is an inverse relationship between the temperature of pasteurization and the whey separation. It is interesting to note, however, that the highest pasteurizing temperature of 175° F. for 30 minutes did not entirely eliminate the trouble.

*The Effect on Whey Separation of Drying and  
Heating the Stabilizer*

To determine specifically if the stabilizer alone contained the causative agent of whey separation, the stabilizer, both in powdered form and in suspension, was subjected to various conditions of desiccation and heat before using in the mix.

A study was made first on the effect of abnormal conditions of drying the powdered stabilizer on the whey separation of the mix. The drying was carried out by using the solids oven of the Mojonnier tester in the manner outlined in the procedure. The results, as shown in table 4 indicate that the whey separation is not retarded by drying the powdered stabilizer for periods as long as 60 minutes. In fact, the few trials of the longer heating periods show the heating to actually increase the whey separation, but this increase may be due to other factors involved in the processing of the mix.

TABLE 3

*The effect on whey separation when the powdered stabilizer was dried at 100° C. in 20 inches of vacuum for varying lengths of time*

SAMPLE	NO. OF TRIALS	LENGTH OF TIME HEATED	PER CENT OF WHEY SEPARATION AFTER		
			24 hrs	48 hrs	72 hrs.
1	6	0 min.	0.8	13	30
2	3	10 "	1.5	14	27
3	4	20 "	3.0	13	26
4	3	30 "	10.0	32	47
5	3	40 "	9.0	34	49
6	2	50 "	2.0	26	46
7	2	60 "		20	39

Since the drying of the powdered stabilizer at 100° C. did not influence the whey separation, it was thought that possibly more efficient means of heating the stabilizer could be obtained if the stabilizer were first put into solution and this solution heated. It appeared logical to assume that if the agent causing the whey separation was present in the stabilizer and was enzymic in nature, it could be inactivated by heating to near boiling

temperature. A method of heating the stabilizer in solution, therefore, appeared to be the most satisfactory.

A water solution of the stabilizer was prepared which contained enough stabilizer to make a concentration of 0.4 per cent in the ice cream mix. The solution was heated to 200° F. to 205° F. for two and one-half hours. It was then cooled and added to the ice cream mix. The mix was processed in a normal fashion and the whey separation observed after varying intervals. The results obtained are shown in table 4.

TABLE 4

*The effect on whey separation when a solution of the stabilizer was heated to a high temperature before adding to the mix*

TRIAL.	PER CENT OF WHEY SEPARATION AFTER			
	24 hrs	48 hrs	72 hrs	96 hrs
1	1.0	2	9	18
2	0.0	5	12	22
3	1.5	9	19	32
4	0.0	0	1	3
5	0.0	0	1	3
6	0.5	5	13	21
7	2.0	3	6	11
8		5	18	27
9		5	18	28
Ave.	0.7	3.8	10.8	18.3

When these results are compared with those in table 3, it may be noted that the heating of the stabilizer in solution retarded the whey separation to some extent. However, the separation of whey was by no means eliminated even with this high heat treatment.

*The Effect on Whey Separation of Heating the Milk Products  
Used in the Ice Cream Mix*

Another possibility of a cause for at least part of the whey separation of the mix is the milk and milk products it contains. In order to determine if some property of the milk, cream, or dried skim milk was aiding or completely causing the "wheying off" of the mix, another pasteurizing procedure was followed.

The mix was prepared as before but without the stabilizer and sugar. It was divided into three lots which were pasteurized for 30 minutes at 145° F., 165° F., and 175° F., respectively. After pasteurizing, the mix was cooled to 145° F. where the stabilizer and sugar were stirred into the respective lots. The mixes were then processed in the usual manner. The per cent of separation of whey as noted after varying storage periods is given in table 5.

TABLE 5

*The effect on whey separation when the milk products were pasteurized at different temperatures before adding the stabilizer (Ave 8 trials)*

LOT	PAST TEMP (°F)	PER CENT OF WHEY SEPARATION AFTER			
		24 hrs	48 hrs	72 hrs	96 hrs
1	145	7.0	26	37	41
2	165	0.3	4	13	22
3	175	0.0	1	5	10

The results of these eight trials show the higher pasteurizing temperatures to bring about a decided decrease in both the rapidity of appearance of whey separation, and in the total amount of whey noted after 96 hours. When the milk products were pasteurized to 175° F for 30 minutes no whey separation was observed in the mix for 48 hours and then the amount was small. In the lots pasteurized at 145° F and 165° F whey separation was observed usually at 24 hours although the amount appearing in the 165° F lot was usually very small or, in some trials no separation was detected.

To determine if the wheying off of the mix could be entirely prevented by heating the milk products, the milk cream, and skimmilk powder were heated to 205° F for one hour. They were then cooled to 145° F and the mixture of sugar and stabilizer added. The mix was further processed as previous mixes. The results are given in table 6.

TABLE 6

*The effect on whey separation when the milk products of the mix were heated to 205° F for one hour*

TRIALS	PER CENT WHEY SEPARATION AFTER			
	24 hrs	48 hrs	72 hrs	96 hrs
1	0.0	0.0	0.00	0.25
2	0.0	0.0	0.00	0.00
3	0.0	0.0	0.00	0.10
4	0.0	0.5	1.00	2.50
5	0.0	0.0	0.00	0.75
6	0.0	0.0	0.25	0.75
Ave	0.0	0.08	0.2	0.72

These results show that the heating of the milk products to an extremely high temperature greatly reduced the tendency of the mix to show whey separation. In only one of the six trials was any whey observed after 48 hours of storage, and in only two trials was any observed after 72 hours of storage. In the majority of the trials a slight amount of whey separation was noted after 86 hours of storage, but in only one (Trial 4) did the separation exceed one per cent.

*The Effect on Whey Separation of Different Percentages of  
Serum Solids in the Mix*

The composition of the mix was varied so that the serum solids content of three lots was respectively 8, 10, and 12 per cent serum solids. The whey separation observed after various storage periods is recorded in table 7.

TABLE 7

*The effect on whey separation when different percentages of serum solids were used in the mix. (Ave. 4 trials)*

PER CENT SERUM SOLIDS	PER CENT WHEY SEPARATION AFTER			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
8	5.0	15	20	25
10	8.0	19	25	31
12	5.0	11	19	30

These results indicate that there is no correlation between the percentage of serum solids of the mix and the amount of whey separation.

*The Effect on Whey Separation of Different Percentages of  
Fat in the Mix*

Maintaining the serum solids content of the mix at 10 per cent, mixes were prepared containing respectively 10, 12, and 14 per cent of fat. The results are given in table 8.

TABLE 8

*The effects on whey separation when different percentages of fat were used in the mix. (Ave. 8 trials)*

PER CENT FAT	PER CENT WHEY SEPARATION AFTER			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
10	42	48	52	54
12	18	22	24	27
14	17	24	26	30

These figures show that the fat may have an influence on the amount of whey separation. A comparatively low fat content of the mix, *i.e.*, 10 per cent, seemed to favor greater whey separation than did a higher percentage of 12 to 14 per cent. However, this did not hold strictly true in all the trials. There was no noticeable difference in the amount of whey appearing in the higher fat mixes.

*The Effect on Whey Separation of Incomplete Cooling of the  
Mix After Pasteurization*

After pasteurization and viscolization, the mix was divided into two lots. Lot I was cooled by ice water to 50° F., while Lot II was cooled only

to 65° F. They were then stored and examined at proper intervals for whey separation. The results are given in table 9.

TABLE 9  
*The effect on whey separation of incomplete cooling of the mix. (Ave. 6 trials)*

LOT NO.	FINAL TEMP. BEFORE STORING (°F)	PER CENT WHEY SEPARATION AFTER			
		24 hrs	48 hrs.	72 hrs	96 hrs
I	50	11	25	44	48
II	65	13	27	45	48

The values shown in this table indicate that the incomplete cooling of the mix had no effect on the whey separation. Undoubtedly, the temperature of the storage room is an important factor in this connection as previously shown (4).

#### SUMMARY

1. Whey separation was observed in samples containing as little as 0.1 per cent of the vegetable stabilizer after 96 hours of storage at 42°–46° F.

2. Using normal amounts of the stabilizer, *i.e.*, from 0.3 per cent to 0.5 per cent, and under the conditions of the experiment, a whey separation of approximately 50 per cent was noted after four days' storage.

3. Pasteurization of the mix was carried out at temperatures of 145° F., 165° F. and 175° F. for 30 minutes. The use of higher temperatures for pasteurization caused less whey separation. After 96 hours of storage the samples pasteurized at 145° F. showed 26 per cent separation, and those pasteurized at 175° F. showed an average of 4.7 per cent whey separation. The higher temperatures also retarded the rapidity with which the whey separation appeared.

4. Drying of the powdered stabilizer at 100° C. and under 20 inches of vacuum for as long as one hour did not affect its property of causing whey separation.

5. Heating a water suspension of the stabilizer to 205° F. for two hours retarded the rate and diminished the total amount of whey separation appearing in the mix when the stabilizer was used. It was, however, not reduced to the extent expected. An average of about 18 per cent of whey was observed after 96 hours of storage.

6. Pasteurization of the milk products to 165° F. or 175° F. for 30 minutes before adding the stabilizer, brought about a reduction in the whey separation which was not greatly different from the results obtained by pasteurization of the products plus the stabilizer at similar temperatures.

7. The greatest reduction in whey separation was obtained when the milk products used in the mix, *i.e.*, milk, cream, and skimmilk powder, were heated to 205° for one hour before the stabilizer was stirred in at 145° F.

By this method the separation of whey was negligible after 72 hours of storage, and showed an average of less than one per cent after 96 hours.

8. The percentage of serum solids in the ice cream mix had no effect on the whey separation.

9. A mix having a low percentage of fat seemed to favor the separation of whey more than a 12 or 14 per cent fat mix.

10. Incomplete cooling of the mix had no influence on the whey separation provided the mix was cooled to about 65° F. after pasteurization.

#### CONCLUSIONS

From the results obtained in this study, it would seem that the principal factor involved in the whey separation is concerned chiefly with the milk products used in the mix, rather than with the stabilizer. There apparently is a reaction, or combination, between the stabilizer used and some natural constituent of the milk, cream, and skinmilk powder to bring about the separation of whey.

This natural constituent of the milk products is, at least partly, heat labile. The whey separation may be due to enzymic action, but this does not appear to be so logical when it is considered that heating of the milk products for an hour did not entirely eliminate the defect. The proteins are probably closely involved with the separation as well as certain salts of the mix.

The elimination of the separation of whey by any practical plant method does not appear feasible. When a stabilizer is used which causes whey separation, the use of high temperatures of pasteurization is perhaps the best means of limiting the extent of the separation.

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# THE NEUTRALIZING POWER OF FORAGE CROPS FOR ORGANIC AND MINERAL ACIDS

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Plant materials differ widely in their chemical composition. In most cases cereals and grasses contain a smaller percentage of basic materials such as calcium, potassium, magnesium and nitrogen and a larger percentage of readily fermentable carbohydrates than do the legumes. These differences in the ratio of the fermentable sugars to the basic constituents in the non-legumes and in the legumes may have a definite effect on the quality of the silage that can be made from these forage crops.

## SUGGESTIONS FROM PREVIOUS WORK

The comparative neutralizing power of non-leguminous and leguminous types of silage materials for organic and mineral acids is unknown. Lemmerman (3) made extracts of Graminae and Leguminosae by boiling the plant roots in water and using a hand press. He observed that three to four times as much barium hydrate was required to neutralize the acids in the extracts of vetch and white clover as was required to neutralize the acids in the extracts of timothy and buckwheat. This shows a fundamental difference between these two types of plant materials.

It was pointed out by Wright and Shaw (7) that both reducing and non-reducing sugars disappear almost completely during the fermentation of both corn and soybean silages and that these sugars are converted into organic acids which preserve the material. Blish (2) concluded that the kind of acids produced was more important than the amount of acids. His data show that the quality of two samples of sunflower silage was good despite the fact that the amount of acids of one was less than one-half that of the other and that a third sample which contained an amount of acids between that of these two completely spoiled. This spoiled sample contained a strong odor of butyric acid while the others did not. The chemical analysis shows that the sugar content of the plant materials which produced the best type of acidity and thus a good silage was seven times that of the sugar crop that completely spoiled. The protein content of these samples was about the same. This may indicate that a wide ratio between the fermentable carbohydrates and the other materials such as nitrogen must be present before good silage can be expected. In fact Blackshaw (1) acted on this suggestion and studied the ratio between the protein and the fermentable carbohydrates of the more common crops grown and used for silage making in Rhodesia and their ability to produce good silage. He

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found that maize which is relied upon to produce good silage surpassed in sugar content all the other crops examined.

Wright and Shaw (6, 7) concluded that the content of fermentable carbohydrates is sufficient to produce the proper degree of acidity in the silage from any green crop, barring a few exceptional cases, and that the only other chemical qualification necessary to a succulent silage is the proper moisture content. They state that any forage crop which is palatable as a forage crop will be palatable as silage if the moisture content is right.

#### A WORKING VIEWPOINT

Some important determinations and points in silage making which have been made and observed by previous investigators suggest a new working viewpoint. It is presented briefly. The successful natural conversion of plant materials into silage is accompanied by the formation of organic acids. As the acidity of the fermenting plant materials approaches a certain pH value the fermentation is automatically retarded. If an insufficient quantity of acid producing carbohydrates is available and the proper degree of acidity fails to develop either from this cause or for any reason such as the neutralizing effect of the plant bases on the acids, putrefaction of the ensiling material may occur to a larger or smaller degree and the quality of the product may be materially reduced accordingly. This suggests that in such a process, cereals, grasses and other non-leguminous plant materials which have been used successfully in making silage contain more sugar and less basic substances like calcium and nitrogen than the legumes such as clover, alfalfa and soybeans which usually produce poor silage or putrify, and that these basic substances in the leguminous materials neutralize and adsorb to a larger extent the acids which are produced in fermentation as fast as they are formed than do the non-leguminous materials. This may indicate that a larger quantity of acids must be produced in leguminous silage than in non-leguminous silage if the degree of acidity of the two materials is to be the same at the end of the fermentation processes. Also, if the first acids produced in a legume silage fermentation are used up in combining with bases it allows considerable time for a certain amount of putrefaction to occur before enough acidity develops to interfere with the putrefactive process. These fundamental differences in the character of the two types of plant materials may result not only in different types of fermentation but also in different qualities of the fermented product.

The acids produced in such fermentations which seem most desirable are lactic, acetic and propionic. The quantity of each acid formed is probably related to the environmental conditions, such as temperature and moisture. Only a certain amount of these acids is ever produced. Apparently their formation is controlled in the silo, where anaerobic conditions largely prevail, by the supply of sugars that the plant materials contain.

## PROBLEM AND METHODS

With the above viewpoint in evidence a study of the neutralizing power of leguminous and non-leguminous forage crops as they relate to the production of silage seemed desirable. Data obtained from such a study are presented in this paper. It is believed that such data may lead to a better understanding of the practices necessary to properly ensile leguminous forage crops. This has a special bearing in New York State where poor weather conditions often prevent the process of hay making from freshly cut material.

Since silage can be made readily from non-legumes and with considerable difficulty from legumes it would seem that a study of this problem in relation to the basic substances that the plant materials contain as measured by their power to neutralize acids may aid in understanding the frequent spoilage of silage due to undesirable fermentations.

A wide variety of crops and plant materials were used in this study in order to obtain a true picture of the neutralizing and adsorbing power of silage materials. They represent both legume and non-legume forage crops. They were obtained at a stage of growth at which they normally would be taken for hay, fodder, silage or seed. After drying they were ground in a Wiley mill so that acids could rapidly penetrate into every particle. One gram portions of the chaffed crop materials were placed in beakers and 25 ml. distilled water added. A definite quantity of acid was then added to the suspensions. Although the action of the crop materials on the acids seemed to be immediate, the pH reading of the mixtures was not recorded until after the suspensions had stood for at least five minutes. Each of the acids was balanced so that 30 ml. would exactly neutralize 30 ml. of a 0.1 normal NaOH solution. This provided equal quantities of acid in each solution. The degree of acidity of the crop materials before and after the addition of the acids was determined by the quinhydrone method. It was assumed that the pH readings thus obtained would indicate the neutralizing and adsorbing capacity of the chaffed one gram portions. It was desired to express the intensity factor of acidity rather than the quantity of acid substances present because it is known that only a few organisms can produce from soluble carbohydrates an intensity factor greater than that expressed by pH 4.5 and because this seems to be near the intensity required to successfully preserve silage materials. It was desired therefore to know how much acid was required to produce an intensity factor near this figure. At the Ontario Agricultural College (4) the colorimetric method was used for pH determinations. The lowest intensity factor of acidity found at any time during the fermentation of sweet clover was pH 4.4 although the freshly cut samples ranged from pH 6.6 to 6.9. This report from the Ontario Agricultural College states that good corn

silages which were tested by this method gave an average intensity factor of pH 4.0.

### RESULTS

The intensity factor of a water-suspension of one gram of each non-leguminous plant material before and after being exposed to 3 ml. of 0.1 normal acids is given in table 1.

TABLE 1  
*Neutralizing power of forage crops for organic and mineral acids*

3 ML N 0.1 ACID	NON-LEGUMINOUS CROP MATERIAL AND pH					
	Corn	Sorghum	Foxtail Grass	Barley Hay	Blue Grass 274	Blue Grass 275
Cheek	5.8	5.7	6.1	6.5	5.6	5.6
Acetic	4.4	4.3	4.5	4.8	4.6	4.6
Citric	4.3	4.2	4.5	4.7	4.4	
Phosphoric	4.2	4.0	4.4	4.7	4.5	4.4
Lactic	3.9	3.8	4.1	4.4	4.0	4.1
Hydrochloric	3.4	3.4	3.8	4.1	3.7	3.6
Sulphuric	3.3*	3.3*	3.7	3.9	3.6	3.6
5 ML N 0.1 ACID	LEGUMINOUS CROP MATERIAL AND pH					
	Vetch	Alfalfa	Alsike Clover	Pea Vines	Pea Flour	Soybean Hay
Cheek	6.0	5.7	5.8	5.7	6.2	5.6
Acetic	4.8	4.6	4.6	4.7	4.5	4.7
Citric	4.6	4.5				4.4
Phosphoric	4.6	4.4	4.4	4.5	3.9	4.6
Lactic	4.2	4.1	4.1	4.3	3.7	4.4
Hydrochloric	4.0	3.8	3.8	4.1	3.3*	4.3
Sulphuric	3.9	3.7	3.7	4.0	3.3*	4.2

\* Estimated from E. M. F.

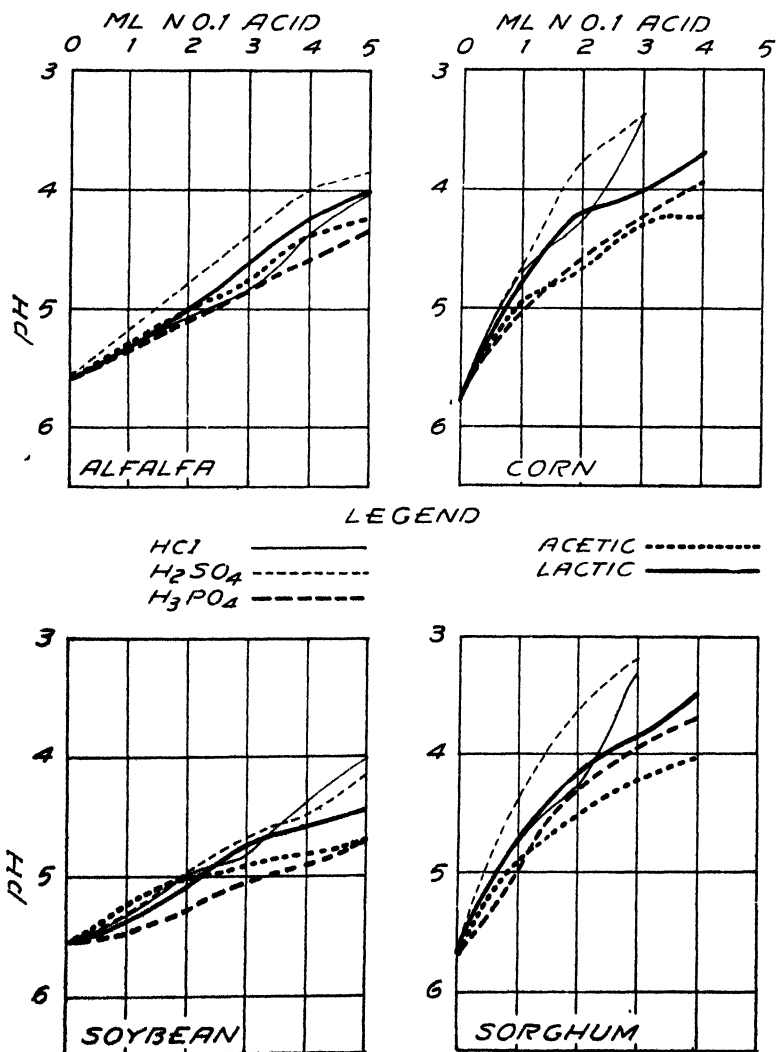
It is evident that plant materials vary considerably in their capacity to neutralize and adsorb organic and inorganic acids. One gram of chaffed corn suspended in 25 ml. of water was changed from pH 5.8 to pH 4.4 by the addition of 3 ml. of 0.1 N acetic acid and to pH 3.3 by the same quantity of 0.1 N sulphuric acid. The effect of the corn suspension on the other acids lies between that of acetic on the one hand and of sulphuric on the other, the order being acetic, citric, phosphoric, lactic, hydrochloric and sulphuric. All non-legume crop materials tested respond to acid treatment in this same order.

Various crop materials did not exhibit the same intensity factor when treated with an identical quantity of acid. A suspension of corn, the reaction of which was pH 5.8, was changed to pH 3.3 by 3 ml. of a 0.1 N  $\text{H}_2\text{SO}_4$  while an oat straw suspension, the reaction of which was normally pH 6.5,

when treated with the same quantity of acid was changed to pH 4.1; and the oat grain of pH 6.2 to an acidity of about pH 2.5.

The intensity factor of water-suspensions of one gram of each leguminous plant material before and after being exposed to 5 ml. of 0.1 normal acid is given also in table 1.

It will be observed that the acidity of these legume crop materials as



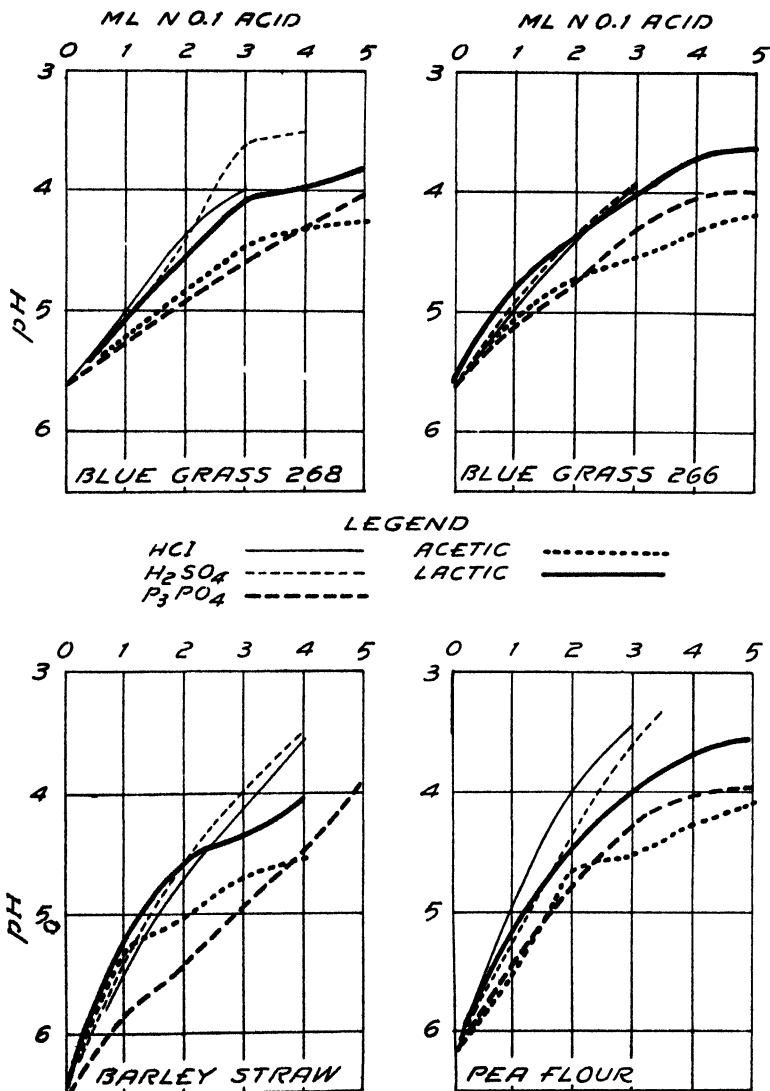
expressed by pH figures before being treated with acids was about the same as that of the non-legumes.

The slightly acid legume crop materials show considerable difference in the intensity of acidity they possess after having been exposed to different acids. A water-suspension of one gram of vetch the acidity of which was pH 6.0 was changed to pH 4.8 by the addition of 5 ml. of 0.1 N acetic acid and a similar quantity of vetch was changed to pH 3.9 by the same quantity of 0.1 N sulphuric acid. The change in acidity of these legume plant materials was increased by the six acids in about the same order as the acidity of the suspensions of the non-legumes was increased but the increase, however, was much less. From the data presented it is difficult to say that the chaffed material of the legumes adsorbed and neutralized more of the citric acid than of the phosphoric acid.

Leguminous and non-leguminous plant materials adsorbed and neutralized larger quantities of organic and mineral acids, as measured by a change in pH values, when they were exposed to larger quantities of the acids. The leguminous materials were more active in this respect than were the materials of a non-leguminous nature. This relationship is shown in graphs 1 to 8. It suggests that the plant materials both adsorb and neutralize the acids. If this were not so it would seem that if the plant materials after having been exposed to acids were then exposed to increasing increments of sodium hydroxide of a strength equal to that of the acids employed, they would neutralize the acid and give the same curve that was obtained when becoming more acid. This apparently does not occur. Chaffed material of Sorghum, the reaction of which was pH 5.7, was treated with 1 ml. increments of N 0.1  $\text{H}_2\text{SO}_4$  until its reaction was about pH 3.3, and a curve thus obtained. After this sodium hydroxide in the same quantities and of the same strength as that of the  $\text{H}_2\text{SO}_4$  was added to the suspension where the pH was 3.3. The suspension did not return to the original but to a much higher pH.

#### DISCUSSION

The data presented in this paper indicate that leguminous crop materials both adsorb and neutralize organic and inorganic acids to a larger extent than do non-leguminous forage materials. In the normal fermentation of leguminous crop materials this is taken to indicate that considerably larger amounts of organic acids must be produced to effect a pH change of 1 unit than would be required if non-leguminous materials were being ensiled. Such a delay in effecting the pH necessary to preserve this material may permit the liberation of a slight quantity of ammonia from the highly nitrogenous material. If this occurs still more acid is required. This beginning of putrefaction, accompanied by the slow decrease in pH may



persist until all of the easily fermentable carbohydrates is utilized. When this situation is reached excessive putrefaction becomes evident and the leguminous forage material that should have produced silage is spoiled and is no longer suitable for animal consumption.

The pH of plant materials whether leguminous or non-leguminous was changed the least by a given quantity of acetic acid and most by the same

quantity of sulphuric acid. This was most pronounced at a pH 4.5 to 3.8, although evident at a higher pH. The order of effectiveness seemed to be acetic, citric, phosphoric, lactic, hydrochloric and sulphuric. The fact that hydrochloric and sulphuric acids are more effective in neutralizing the basic nature of plant materials, whether leguminous or non-leguminous than acetic, citric, phosphoric or lactic is probably the reason why Virtanen (5) recommends the employment of these acids in the artificial preservation of highly nitrogenous forage crops.

Whether these acids can be profitably used to augment or supplant the normal formation of acids in silage production is an economic question that still deserves attention. The difficulties encountered in making hay of such crops as the first cutting of alfalfa together with the losses that often occur emphasize the importance of such an undertaking.

#### SUMMARY AND CONCLUSION

A study was made of the ability of forage crops to neutralize organic and mineral acids. Such crops as alfalfa, vetch, timothy, barley, oats, corn and bluegrass were employed. Finely ground portions of such crops were exposed to acids of known strength and any change in the intensity factor of the material noted.

It was observed that leguminous materials require more acid to bring about a change of 1 pH unit than was required by non-leguminous materials and that more acid was required by both types of material to change their reaction 1 unit in the region of pH 4.0 than was required in the region of pH 6.5. The strong mineral acids were more effective in this respect than were the organic acids.

From the data presented it seems doubtful whether certain leguminous materials contain enough fermentable carbohydrate material if it were converted quantitatively into organic acids to produce an intensity factor comparable to that found by other investigators in the good types of silage which have been produced from crop materials containing a larger percentage of fermentable sugars. It is concluded that this is probably the main reason why many failures have been recorded in attempts to produce silage from leguminous crops.

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# THE DETECTION OF FORMALDEHYDE IN MILK BY MEANS OF THE METHYLENE BLUE REDUCTION TEST<sup>1</sup>

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That the addition of formaldehyde to milk decreases the bacterial content and prolongs the keeping quality has been known by dairymen for many years. Although suitable tests are available for the detection of formaldehyde in milk, these tests are not usually made unless the suspicion of the purchaser has been aroused.

Schardinger<sup>2</sup> observed that the addition of a formaldehyde-methylene blue solution to milk caused a rapid decolorization of the dye to its leucobase. From Schardinger's observations it is easy to deduce that milk to which formaldehyde has been added should likewise show a short reduction time when subjected to the routine methylene blue reduction test. Inquiry at several milk receiving stations where the methylene blue reduction test was employed confirmed this deduction and revealed that confusion had frequently arisen from analyses of individual samples of milk which showed a short reduction time (less than 30 minutes) and at the same time, a low plate count. Analyses were made for formaldehyde in several samples showing such anomalous results, and in each case the test was found to be positive for this preservative.

The wide-spread use of the methylene blue reduction test in dairy plants suggests the possibility that this test may offer a practical means of identifying the samples of milk to which formaldehyde has been added. A short reduction time certainly could not be regarded as convincing evidence that formaldehyde had been added to milk, but it might serve as a basis for more intelligent selection of the samples to which sensitive tests for formaldehyde were to be applied.

## METHODS

Several preliminary experiments were performed to determine the general range of dilutions of formaldehyde which affects the bacterial count and the reduction time of milk. The methods employed and the results obtained in these preliminary experiments were essentially the same as those of the final experiment presented in this paper.

By suitable serial dilution, concentrations of formaldehyde ranging

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<sup>2</sup>Schardinger, F. 1902. Über das Verhalten der Kuhmilch gegen Methyleneblau und seine Verwendung zur Unterscheidung von ungekochter und gekochter Milch. *Zeit. Unters. Nahr. Genuss.* 5: 1113-1121.

from 1:100 to 1 1000,000 were prepared. (These concentrations are calculated on a basis of actual  $\text{HCHO}$  and not as commercial formalin). Standard plate counts were made on the raw milk before it was treated with formaldehyde. Immediately after the various dilutions of formaldehyde had been prepared duplicate methylene blue reduction tests were set up. After the milk had been in contact with the formaldehyde for 24 hours at  $21^{\circ}\text{C}$ ., standard plate counts and methylene blue reduction tests were again made.

#### RESULTS

The data in the accompanying table show the effect of various concentrations of formaldehyde on the bacterial growth, reduction time, coagulation time, and taste of the milk.

*Bacterial growth.* The standard plate count on the original milk was 18,000 per cc. After 24 hours at  $21^{\circ}\text{C}$ . the milk containing formaldehyde in concentrations of 1:2500 or more showed no colonies on 1 in 10 dilution plates. The numbers of bacteria were markedly decreased by concentrations of formaldehyde up to 1:20,000, slight growth was evident in the presence of 1 25,000, and appreciable growth occurred in the presence of higher dilutions of formaldehyde. As may be seen from the results in the table, the standard plate count increased in 24 hours from 18,000 to 470,000 per cc. in the presence of a 1:30,000 concentration of formaldehyde. Within the limitations imposed by observations of a single experiment, these results suggest that the addition of formaldehyde in concentrations of less than 1:25,000 failed to retard bacterial growth sufficiently to keep the count below the usual bacterial standards for market milk. Results of experiments not included in this paper indicate that the minimum concentration of formaldehyde required to inhibit growth varies with different samples of milk, and in general the higher the bacterial content of the milk the higher the concentration of formaldehyde required.

*Methylene blue reduction time.* The reduction time observed for the original sample of milk without formaldehyde was 660 minutes; the addition of 1:100,000 concentration of formaldehyde shortened the reduction time to 259 minutes. Similarly, increasing quantities of formaldehyde shortened the reduction time progressively to as low as 21 minutes in the case of a 1:1000 concentration. However, when a 1:250 concentration of formaldehyde was added the reduction time was 440 minutes and a 1:100 concentration inhibited reduction.

When formaldehyde was added to this milk in concentrations between 1:1000 and 1:10,000 the reduction time was decreased to a few minutes. It is apparent that the short reduction times observed in the presence of 1:1000 to 1:10,000 concentrations is largely, if not wholly, the result of chemical rather than of biological factors. When sufficient formaldehyde

*The effect of formaldehyde on the bacterial count, reduction time, coagulation time and taste of milk*

DILUTION OF FORMALDEHYDE 1:	BACTERIAL COUNT		REDUCTION TIME (MINUTES)		TIME (HOURS) OF COAGULATION WHEN INCUBATED AT		PERCENTAGE OF PERSONS DETECTING FORMALDEHYDE BY TASTING THE SAMPLE
	Before adding formaldehyde	After 24 hrs. at 21° C.	Immediately after adding formaldehyde	After 24 hrs. at 21° C.	21° C.	37° C.	
100	18,000	0	#	#	X	X	100
250	18,000	0	440	#	X	X	100
500	18,000	0	24	510	X	X	100
750	18,000	0	22	55	X	X	100
1000	18,000	0	21	22	X	X	100
2500	18,000	0	23	29	X	X	90
2500	18,000	120	33	38	284	360	80
7500	18,000	460	40	64	188	180	30
10,000	18,000	830	45	125	172	140	50
15,000	18,000	2,000	55	183	188	92	10
20,000	18,000	2,200	72	168	188	48	20
25,000	18,000	77,000	90	140	164	53	10
30,000	18,000	470,000	99	162	116	53	20
40,000	18,000	1,300,000	132	254	130	48	10
50,000	18,000	73,000,000	182	42	100	48	20
75,000	18,000	90,000,000	225	36	124	48	30
100,000	18,000	140,000,000	259	34	116	48	20
0	18,000	120,000,000	660	50	100	48	20

# = not reduced in 24 hours.

X = not coagulated after 220 days.

was added to prevent effectively rapid bacterial development, (1:25,000 or more) the reduction time was so greatly shortened that it should arouse suspicion, especially if plate counts or keeping quality tests were also applied. Even the addition of relatively ineffective quantities of formaldehyde (1:30,000 and less) shortened the reduction time to values which were out of harmony with the plate counts.

The tendency for formaldehyde to induce rapid reduction was less pronounced after 24 hours. This suggests that the fall in potential prerequisite to reduction of the dye is delayed either by the removal of part of the formaldehyde added, as a result of interaction with milk constituents, or by the formation of substances which tend more effectively to poise the oxidation-reduction system. However, this does not completely destroy the value of the reduction test as an indicator of the addition of formaldehyde. Within the range of concentrations of formaldehyde most likely to be employed (1:15,000 to 1:25,000) the reduction times after 24 hours were between 183 and 140 minutes although the plate counts were between 2000 and 77,000 per cc. Thus it is evident that the anomalous relationship between the plate counts and reduction time persisted after the milk had been held for 24 hours.

*Coagulation time* Two additional sets of milk samples containing the dilutions of formaldehyde indicated in the table were prepared as previously described. One set was placed at 21° C and the other at 37° C.; these were observed at frequent intervals for the first evidence of coagulation. The data in the table show that concentrations of formaldehyde of 1:25,000 or more markedly delayed coagulation at both temperatures. When formaldehyde in concentrations of 1:1000 or more was added coagulation was indefinitely delayed (more than 220 days).

*Detection of formaldehyde by taste.* A set of 17 samples containing the concentrations of formaldehyde indicated in the table and 6 control samples containing no formaldehyde were prepared. These 23 samples were arranged and numbered at random. Ten persons tasted these samples and recorded the presence or absence of a formaldehyde taste. Four of the people were actively engaged in the judging of dairy products. The remaining 6 might be classed as ordinary consumers who were not specially trained in the tasting of dairy products.

Before tasting the regular series of numbered samples, each person was required to taste 3 special samples of milk, one of which contained no formaldehyde, one tasted strongly of formaldehyde (1:500), while in the third the formaldehyde was barely detectable by taste. The 10 people who assisted in the tasting were instructed to practice on the special samples until they could differentiate correctly the known samples. All tasting of the unknown samples was done without knowledge or suggestion as to the formaldehyde content and without knowledge of the findings of others who

had tasted the samples. The taste of formaldehyde was reported by some in control samples, especially where a control followed a sample containing a high concentration of formaldehyde. There were many such inconsistencies in the results, due to personal differences and to the carrying over of the taste from one sample to another.

The data in the table show that when formaldehyde was present in concentrations of 1:10,000 or more, it was detected rather consistently by all who tasted the samples of milk. In concentrations of 1:15,000 and less, only 10 to 30 per cent of the tasters detected it. The significance of the ability to detect formaldehyde in very high dilutions is diminished by the fact that 6 of the 10 people reported formaldehyde in 3 of the 6 control samples. Out of the 6 control samples tasted by 10 persons there were 8 cases (13.3 per cent) in which formaldehyde was erroneously reported.

#### SUMMARY DISCUSSION

It is possible to use the methylene blue reduction test as a basis for suspecting the presence of formaldehyde in milk. The addition of effective quantities of formaldehyde shortens the reduction time, especially if the methylene blue reduction test is run soon after the formaldehyde has been added. It is not intended to imply that the methylene blue reduction test can be used as a specific test for the presence of formaldehyde, but that short reduction times may suggest the application of more exact qualitative tests for the presence of formaldehyde.

The range of dilutions of formaldehyde which effectively retarded bacterial growth, definitely delayed the coagulation time, and at the same time escaped detection by a majority of those who tasted the milk was between 1:15,000, and 1:25,000. The addition of more than 1:15,000 formaldehyde is quite certain to be detected by the consumer, whereas less than 1:25,000 fails to accomplish the purpose for which it is usually added. In other words, the dairyman must be able to adjust the final concentration within this rather narrow range. On a basis of the volume of commercial formalin the margin between "enough" and "too much" is only 0.25 cc. or about 5 drops per gallon. The chemical precision requisite for such careful adjustment is not usually to be expected outside of the laboratory. This suggests that the addition of formaldehyde to milk by the average dairyman is more likely to fail than it is to accomplish the intended result. The addition of too much is likely to be detected by the consumer or to arouse the suspicion of the milk plant operator by anomalous results of the methylene blue reduction test as outlined in this paper. Specifically, any sample of fresh milk which shows a reduction time of less than one hour may be suspected of containing formaldehyde, especially if the keeping quality or the bacterial counts are contraindicative.



## PRELIMINARY OBSERVATIONS ON CERTAIN SEASONAL VARIATIONS IN THE PHYSICAL PROPERTIES AND NUTRITIVE VALUE OF COW'S MILK SERUM

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In our work on the problem of zinc in the nutrition of the rat (unpublished data) considerable difficulty was encountered in supplementing the basal synthetic ration with preparations which were potent in the water-soluble vitamin fraction. The basal ration, because of its high purity with respect to zinc, was deficient in all of the water soluble vitamins except B<sub>2</sub>, which was sufficiently supplied in 15 per cent of dried egg white. When alcoholic extracts of yeast or liver together with an extract of pork muscle, rich in vitamin B<sub>1</sub>, were used to supply the remaining water-soluble factors, poor growth of the animals resulted. It was found that when 2 cc. of whole milk were administered daily to the animals a favorable growth response was obtained. This amount of milk, however, introduced considerable zinc, so an attempt was made to obtain the vitamin B complex from milk and at the same time remove the zinc.

A procedure was developed for the preparation of this fraction from skimmed milk. The skimmed milk was obtained from the University dairy and was an average grade of market milk coming from about twenty herds of cattle. Six gallons of the skimmed milk were placed in a porcelain jar, the temperature brought to 40° C. and 10 cc. of rennet added. After ten minutes the curd was carefully cut and the temperature slowly raised to 46° C., thereby producing a tough curd which settled to the bottom. The resulting whey, which was clear and free from casein particles, was drawn off and allowed to stand at room temperature for 24 hours, permitting the lactic acid organisms to lower the pH to the isoelectric point of the albumin (pH 4.5). It was then placed in a two-liter Erlenmeyer flask and heated in a boiling water bath with frequent stirring until the temperature reached 88° C., which required about 15 minutes. The hot material was cooled on ice and the albumin permitted to settle. The clear, cool serum was siphoned off and concentrated ammonium hydroxide slowly added until a precipitation of the phosphates occurred (pH 9). This precipitate, which contained all but a trace of the remaining zinc, settled to the bottom. The supernatant liquid was withdrawn, and by addition of acetic acid the pH lowered

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to 6. The serum was a clear yellow solution which exhibited a green fluorescence. It had a pleasing odor and taste and was readily consumed by the animals.

About the first of February, 1934, a change had occurred in the milk from which the serum was prepared. At this time it became impossible to obtain a clear yellow fluorescent solution upon the precipitation of the albumin. When ammonium hydroxide was added the resulting phosphate precipitate was obtained with more difficulty and was somewhat slower in settling to the bottom. The serum, when brought to a pH of 6, retained a cloudy appearance in contrast to the clear yellow solution which was obtained before the milk had undergone this change. The milk remained in this condition until about the first week of June, 1934, or until the cows had been placed on pasture. The change to green feed had imparted to the milk some change which allowed the preparation of a clear zinc-low serum.

Another series of experiments was started the first week of October, 1934, with the hope of finishing the experiment before the milk changed to the winter condition. It, however, happened that in this year the milk underwent the change to a winter condition in the last week of October, or about 3 months earlier than in the preceding year. The severe drought of the summer of 1934 had limited very seriously the amount of green pasture for the herds.

The nutrition experiments were continued for 12 weeks on the winter serum. It was therefore possible to compare the growth of the control animals, which had received zinc in the summer serum experiments, with the control animals which received zinc in the winter serum experiments. Since the rations were the same in each case any differences in the rate of growth of the animals could therefore be attributed to the serums from the two seasons.

There were 8 animals on the control ration which received summer milk serum—4 males and 4 females, 9 animals on the winter serum experiment—6 males and 3 females. The animals on the summer serum made an average gain of 111 grams for the first 8 weeks, while those receiving the winter serum gained 89 grams in the same period. See Chart 1.

The fact that the winter milk serum was lower than the summer milk serum in certain nutritional factors was also exhibited in another way. Animals which were placed on the zinc low diet for 6 weeks practically ceased growing. If zinc was then administered there was a strong impulse to grow. Animals on summer milk serum made gains of 16 to 22 grams per week for the 4 weeks following the administration of zinc, while those on winter milk serum made gains of 7 to 12 grams for the same period. See Chart 1. These results are in agreement with those reported by Elvehjem, Hart and others (1), who found that rats on a whole milk diet supple-

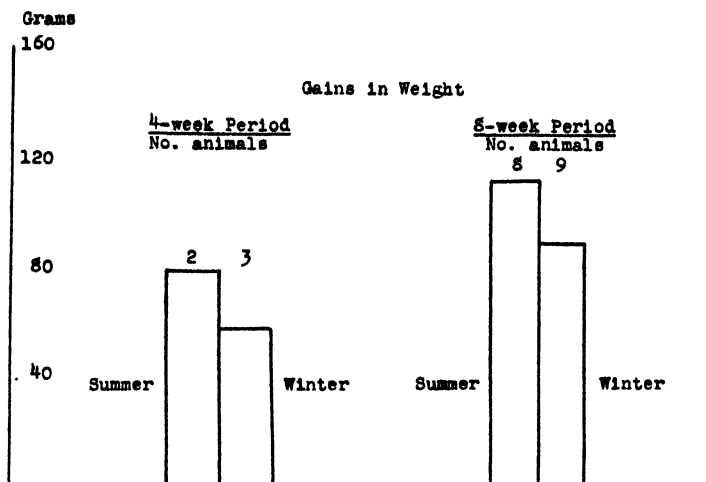


CHART I

Summer and Winter milk serums as supplements to purified rations.

mented with copper, iron, and manganese failed to grow as well on milk produced in the late winter months as on milk produced in the early fall.

Each year when the commercial milks showed the change to the winter condition, milk was obtained from animals of the University herd, which received choice rations of alfalfa hay and corn silage. This milk also changed in its physical behavior at the same time as the commercial milks from the University dairy. Milk from animals receiving artificially dried roughages also exhibited the same physical behavior as the winter commercial milk.

Numerous attempts were made to obtain from these winter milks a clear serum which would resemble that from summer milk. Experiments were made where different salts were added to the whey in varying amounts before the precipitation of the albumin. The salts tried were potassium phosphate (dibasic), sodium citrate, magnesium sulfate, and calcium lactate. One preparation of clear serum was obtained upon the addition of 0.2 gram of calcium lactate per liter of whey. This, however, could not be repeated with the same beneficial effects. It was found later that if the pH of the whey were reduced from 4.5 to 4 by allowing the whey to stand at room temperature for 48 hours instead of 24 and then increased to 4.5 by the addition of ammonium hydroxide, good precipitation resulted. This procedure has not always been found to work satisfactorily for the production of a clear serum from winter produced milk.

It is not possible for us at the present time to state definitely what constituents have been altered in winter milk causing this change in its physical

properties and the decrease in the nutritive value of the serum. The poor growth promoting qualities of winter serum are in accordance with the results obtained when rats are fed mineralized winter milk as the sole diet. This work indicates that the decrease in growth promoting properties of winter milk is due to a deficiency of the water-soluble vitamins in the serum.

It is generally, but not universally, accepted that vitamin B<sub>1</sub> is constant in cow's milk due to its synthesis by intestinal bacteria (2). In respect to B<sub>2</sub> Hunt and Krauss (3) have shown that its level in cow's milk varies with the ration and is highest when the animal is on green pasture. There are no data available in respect to B<sub>4</sub>.

#### SUMMARY

1. The serum of winter produced milk showed physical characteristics at variance with that of summer produced milk. It was clarified with greater difficulty. An adjustment of the salt balance and pH improved somewhat the technique used for the preparation of the serum from winter milk. The time at which these changes occur varies from year to year.

2. The serum of winter produced milk showed lower nutritive value than that of summer produced milk as measured by its use in supplementing a highly purified diet as a source of the vitamin B complex.

3. The significance of these studies lies in the relation of fresh plant tissue as contrasted with field dried material to subtle changes in the milk secreted.

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## THE DEVELOPMENT OF NUTRITIONAL ANEMIA IN DAIRY CALVES

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### INTRODUCTION

During recent years experimental work has definitely shown that whole milk is not a complete food for certain species of animals. A review of the literature has revealed that the amount of such work reported with calves when this experiment was begun was meager. The experiments reported have shown that attempts to raise calves on milk exclusively have usually ended disastrously. After 6 or more months of such feeding the calves would experience convulsions, finally succumbing during one of the spasms. In view of the strikingly beneficial results obtained with some species through the addition of iron and copper to an exclusive milk diet, it was felt that additional information of scientific interest might be obtained by studying the response of dairy calves to a similar treatment, particularly since whole milk or skimmilk constitutes such a large portion of the ration of calves during the first few months of life.

### REVIEW OF LITERATURE

It has long been recognized that whole milk is deficient in iron. The addition of inorganic iron to an exclusive whole-milk diet will not take care of this deficiency in rats (10, 15), pigs (5), or rabbits (10). Later, Hart and coworkers (11) found that, by adding both iron and copper to a whole-milk diet, nutritional anemia in the rat could be prevented or cured. This was found later to apply also to pigs (6).

According to calf feeding experiments (3, 17) in which whole milk was fed as the sole constituent of the diet, malnutrition or death usually ensued after several months of such feeding. Recent experiments indicate that probably this was due to vitamin and mineral deficiencies.

Dairy calves have a definite requirement for vitamin D. Bechdel, Landsburg, and Hill (1), Huffman (12), and Rupel, Bohstedt, and Hart (21) agree that calves will develop rickets on a diet low in vitamin D and can then be cured by supplementing such a diet with vitamin D.

According to Mead and Regan (19) dairy calves have a definite requirement for vitamin A, as well as for certain minerals found in the ash from alfalfa hay. Converse and Meigs (4) also found, by supplementing a poor quality skimmilk diet with cod liver oil, that nearly normal growth resulted without vitamin A deficiencies appearing in the calves.

Cannon (3) found that a whole-milk diet supplemented with cod liver oil and bone meal would furnish sufficient nutrients for dairy calves to an age somewhat over 220 days. After that it was necessary to supply the calves with alfalfa hay or oat straw in order to prevent death from nutritional anemia.

#### PLAN OF EXPERIMENT

This experiment was conducted with 12 Holstein male calves, divided into two series of six each. All calves were started on the experiment at about 4 days of age.

The first series of six calves was started in 1931 and finished in 1932. The calves were grouped in pairs according to the erythrocyte and hemoglobin content of their blood, as determined when they were 2 days of age. The calf in each pair having the higher erythrocyte count and hemoglobin content was placed in Lot I (Calves M 360, M 363, and M 364), while the calf having the lower erythrocyte count and hemoglobin content was placed in Lot II (Calves M 361, M 362, and M 365).

Whole milk collected at random from a Holstein and Jersey herd was fed twice daily. The maximum amount of milk fed to each calf was regulated according to the consumption of the poorest eater of the series.

Each of the Lot II calves received, in addition to the whole milk, 400 mg. of iron, as ferric chloride, and 40 mg. of copper, as copper sulfate, daily, except Sundays and holidays.

The second series of calves was started in 1933 and finished in 1934. They were divided into Lots I (M 406, M 408, and M 414) and II (M 407, M 411, and M 416) as in the first series, except that the first available calf was placed in Lot I and the next available calf (pair mate) was placed in Lot II, but not on the basis of erythrocyte and hemoglobin values. They were fed whole milk twice daily, at the rate of 1 pound to every 10 pounds of live weight. However, at times some calves would refuse part of their allotted milk. The milk used in this series came from an equal number of Holstein and Jersey cows receiving a ration containing alfalfa hay, corn silage or wet beet pulp, yellow corn, oats, wheat bran, linseed oilmeal, and salt.

The Lot II calves were fed daily, in addition to the whole milk, iron and copper supplements according to live weight; *i.e.*, they were given daily, except Sundays and holidays, 400 mg. of iron, as ferric chloride, and 40 mg. of copper, as copper sulfate, until they weighed 200 pounds. Subsequently, for every 100 pounds additional gain in live weight the iron was increased 200 milligrams and the copper 20 milligrams, so that a calf weighing 200 to 300 pounds received 600 milligrams of iron and 60 milligrams of copper.

The calves were located away from sunshine in the calf barn. Wood

shavings were used as bedding. All calves were muzzled throughout the experiment, except at feeding time. Muzzles made from  $\frac{1}{8}$ -inch-mesh heavy screen were used in Series I; those used in Series II were made from 0.040-inch pyrolin-viscoloid (a celluloid-like compound). Each pair of calves remained on the experiment until the death of a pair mate or until approximately 8½ months of age. Several times during the experiment all the calves were treated for lice with a 2½ per cent creolin solution.

The number of erythrocytes and the hemoglobin values of the blood were determined when the calves were one and 3 days of age and approximately every 2 weeks thereafter. Histological studies of the erythrocytes were made from time to time. Live weights were obtained at birth and every 2 weeks throughout the experiment. Measurements of the height at withers were made every 2 weeks during the last 3½ months of the first series, and every 2 weeks throughout the experiment during the second series. Post-mortem examinations were made. Iron, copper, and vitamin A were determined on the livers of the Series II calves.

#### METHODS OF ANALYSES

Samples of freely flowing blood for red cell counts were obtained from the median ear vein, by the use of a Trenner automatic diluting pipette. The samples were diluted 200 times with 1 per cent sodium chloride solution. About a drop of this diluted blood was then placed in a Levy-Hauser counting chamber and the number of red cells per cubic millimeter of blood was estimated. Another sample of freely flowing blood for hemoglobin determination was taken and diluted 200 times with approximately 1 per cent hydrochloric acid. After standing for 30 minutes in a warmed room the grams of hemoglobin per 100 cc. of blood were determined in a Bausch and Lomb hemoglobinometer (improved Newcomer model). The condition of the red blood corpuscles was studied by preparing blood films from the calves fed milk exclusively (Series II) when they were very anemic. Similarly, blood cells from their pair mates (fed milk, iron, and copper) were studied at corresponding ages. The blood films were fixed with mercuric chloride and stained with eosin-methylene blue.

Livers taken from the calves in Series II were saved and frozen until a convenient time for analysis for iron, copper, and vitamin A. Iron was determined by the Stugart (22) method and copper by the sodium diethyldithio-carbamate method, according to Williams (23). Vitamin A determinations on the livers were made with the antimony trichloride reagent.

#### RESULTS

##### *General Health*

*Series I (1932).*—The calves were healthy and vigorous during the first 150 days of the experiment as far as external appearances were concerned.

During the remaining period of 105 days convulsions were frequent, except in calves M 364 (Lot I) and M 361 (Lot II). Scours were very prevalent at times, except in M 364.

External signs of malnutrition were evident toward the latter part of the experiment. There was gradual enlargement of the knees and slight stiffness of the legs resembling rickets. Calves M 363 (Lot I) and M 365 (Lot II) were first to become stiff and emaciated. Respiration was rapid. This occurred when they were about 180 days of age.

All calves but one in this series were alive at the close of the experiment (255 days). M 360 (milk-fed) died at the age of 254 days. This calf appeared healthy on the morning of its death, which occurred during the only convulsion observed in this animal.

*Series II (1934).*—The calves fed whole milk exclusively (Lot I) appeared healthy externally during the first 120 days of the experiment. For the remaining period of 75 days Calves M 406 and M 414 were rough in the hair coat and scoured very profusely at times. M 406 died when 196 days of age and M 414 was killed on the 194th day because of his extremely poor condition. Convulsions were noted in M 406 at 171 days and 183 days of age. One convulsion was noticed in M 414 at 143 days of age. Calf M 408 remained fairly healthy throughout the experiment except for failing appetite from 162 days of age to the end of the experiment (255 days). All three calves were pale about the muzzle and eyes and rough in the hair coat when they were anemic.

The calves fed milk, iron, and copper (Lot II) remained healthy throughout the experiment, except for two cases of scours, a condition which was prevalent in the general calf herd.

### *Erythrocyte and Hemoglobin Data*

*Series 1 and 2 combined.*—Results of the erythrocytes and hemoglobin determinations for each pair of calves are given in table 1. The combined average of all determinations of red cell counts for the calves fed whole milk exclusively (Lots I) is  $8.59 \pm 0.10$  millions per cubic millimeter of blood and for the calves fed milk, iron, and copper (Lots II)  $9.78 \pm 0.09$  millions, or a difference of 1,190,000 cells. Owing to a shrunken and broken condition of the erythrocytes (See Figs. 1 and 2) at the time the hemoglobin reached the level of 5 to 6.5 grams, the cell counts are probably unreliable for the milk-fed calves. The same cell condition existed in the milk-fed calves of Series I; however, no complete records were kept. The average amount of hemoglobin per 100 cc. of blood for the exclusive milk-fed calves is  $7.62 \pm 0.17$  grams, which compares very closely with that of "salt sick" yearling cattle (8.38 gm.), as reported by Neal and Becker (20). The average hemoglobin value for calves fed milk, iron, and copper is  $10.52 \pm 0.10$  grams. This lower value, as compared with that for mature

TABLE 1  
Red blood corpuscles and hemoglobin data\* (Calves arranged according to pairs)

AGE	LOT I				LOT II				LOT I				LOT II			
	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.
SERIES I (1932)																
	M 360				M 361				M 363				M 362			
2 days	10.37	13.2	7.94	11.2	14.53	11.0	8.29	8.7	11.57	12.2	6.78	8.1	11.57	12.2	6.78	8.1
4 wks.	10.61	9.8	10.38	10.1	8.76	11.6	8.69	10.0	10.75	10.4	9.49	9.8	10.75	10.4	9.49	9.8
8 wks.	9.12	10.0	9.65	10.0	7.12	10.0	11.16	11.0	7.81	8.1	9.16	11.0	7.81	8.1	9.16	11.0
12 wks.	10.26	9.1	12.90	12.4	10.06	10.1	10.32	11.8	8.17	8.0	12.20	10.3	8.17	8.0	12.20	10.3
16 wks.	10.94	9.8	10.56	12.2	10.03	10.1	11.47	12.0	10.11	9.0	11.33	11.5	10.11	9.0	11.33	11.5
20 wks.	9.51	5.6	11.90	12.0	8.04	8.9	9.38	14.2	7.58	7.0	11.32	13.4	7.58	7.0	11.32	13.4
24 wks.	8.47	5.6	13.26	12.1	7.98	9.2	8.0	9.8	6.82	7.0	10.50	10.2	6.82	7.0	10.50	10.2
28 wks.	8.38	7.0	9.98	11.4	8.50	9.5	10.35	13.5	7.35	5.5	10.54	12.6	7.35	5.5	10.54	12.6
32 wks.	8.63	8.8	8.72	9.2	7.84	9.0	10.74	11.4	7.34	7.0	9.81	9.9	7.34	7.0	9.81	9.9
35 wks.	8.82	7.7	9.04	9.8	8.36	10.5	11.15	12.0	6.52	6.8	8.10	9.5	6.52	6.8	8.10	9.5
37 wks.	—	—	7.87	8.5	6.63	8.8	8.78	9.8	6.04	6.3	—	—	6.04	6.3	—	—
SERIES II (1934)																
	M 406				M 407				M 408				M 411			
2 days	7.66	8.8	8.48	9.7	12.55	12.0	8.26	8.7	8.63	11.4	8.98	11.9	8.63	11.4	8.98	11.9
4 wks.	8.48	6.4	8.74	10.0	10.70	13.0	10.50	10.2	8.69	5.8	10.09	12.6	8.69	5.8	10.09	12.6
8 wks.	9.12	4.8	10.27	11.5	12.43	8.8	10.23	9.8	8.75	6.3	8.35	10.5	8.75	6.3	8.35	10.5
12 wks.	8.71	5.3	9.73	9.3	8.90	7.9	8.93	9.5	5.88	3.4	8.23	8.6	5.88	3.4	8.23	8.6
16 wks.	9.09	4.0	9.10	9.7	8.93	6.5	8.35	8.7	5.23	3.3	7.16	8.3	5.23	3.3	7.16	8.3
20 wks.	7.87	3.1	8.95	10.8	7.13	4.5	8.71	8.4	6.10	3.2	7.19	9.3	6.10	3.2	7.19	9.3
24 wks.	9.18	4.1	7.62	8.8	6.97	4.0	7.89	8.5	9.44	4.1	9.20	7.3	9.44	4.1	9.20	7.3
28 wks.	—	—	—	—	6.93	3.4	6.64	8.0	—	—	—	—	—	—	—	—
32 wks.	—	—	—	—	6.44	4.5	7.19	8.0	—	—	—	—	—	—	—	—
37 wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

\* The Lot I calves received whole milk exclusively and the Lot II calves received whole milk with iron and copper supplements.

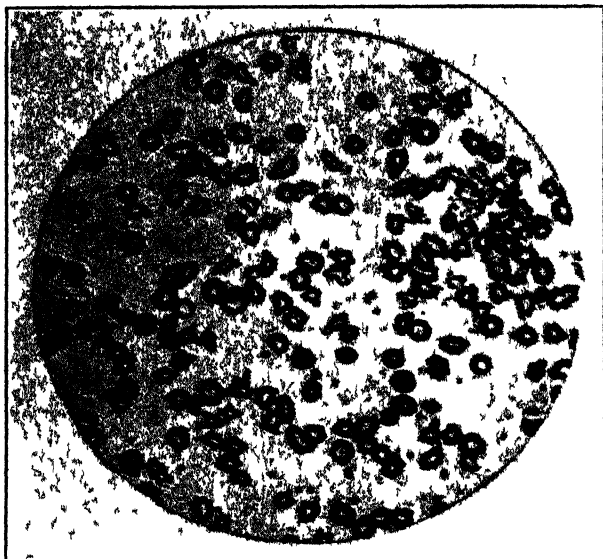


FIG 1 PHOTOMICROGRAPH OF A BLOOD SMEAR FROM A MILK FED CALF OF SERIES II SHOWING CONDITION OF RED CELLS AT TIME OF SEVERE ANEMIA ( $\times 550$ )

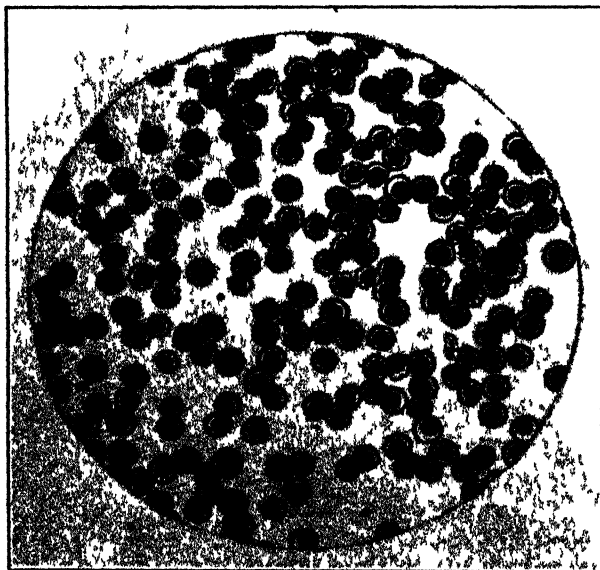


FIG. 2 PHOTOMICROGRAPH OF A BLOOD SMEAR FROM A CALF FED MILK, IRON, AND COPPER OF SERIES II (PAIR MATE TO ABOVE) SHOWING CONDITION OF RED CELLS ( $\times 550$ )

bulls of  $11.92 \pm 0.26$  gm. (2) and  $12.8 \pm 0.8$  gm. (18), indicates that the concentration of hemoglobin in the blood increases as the rate of growth decreases with advancing age. Table 1 shows that the hemoglobin values for the calves of Lots II increased soon after birth and then gradually decreased with advancing age, indicating that as the rate of growth increases the concentration of hemoglobin tends to decrease.

## GROWTH

The average rate of gain in live weight for the Lot I and II calves in Series I was practically the same for the first 224 days. During the remaining 31 days those in Lot I decreased slightly in live weight, while those receiving whole milk, iron, and copper (Lot II) continued to increase in weight. The average live weight at the close of the experiment (255 days) for the Lot I calves was 363 pounds and for the Lot II calves 430 pounds. A similar situation was found in the second series. Average live-weight gains were about the same for both lots until they were 112 days of age. At about 196 days of age the Lot I calves averaged 353 pounds; whereas the Lot II calves averaged 517 pounds.

TABLE 2  
*Weights of organs and color of tissues*

CALF NO	WT OF TESTES GM	WT OF SPLEEN CM	WT OF LIVER GM	WT OF HEART GM	COLOR OF LIVER	COLOR OF MUSCLE TISSUE
Lot I*						
M 360	91	599	1973	885	Very pale	Very pale
M 363	91	329	2086	748	Slightly pale	Slightly pale
M 364	159	476	2472	839	Pale	Pale
M 406	91	181	2403	997	Very pale	Very pale
M 408	227	612	3809	1723	Pale	Pale
M 414	45	227	3084	907	Pale	Pale
Average	117	402	2638	1016		
Lot II*						
M 361	238	749	2222	1089	Dark red	Dark red
M 362	227	635	2086	816	Dark red	Red
M 365	181	340	1587	748	Dark red	Red
M 407	159	1088	5442	1406	Dark red	Dark red
M 411	281	862	5669	1723	Dark red	Dark red
M 416	141	794	2648	1224	Red	Red
Average	204	744	3275	1167		
Per cent Diff.	42.6	45.9	19.4	12.9		

\* Lot I received whole milk exclusively and Lot II received whole milk with iron and copper supplements.

A slower rate of growth in height at the withers was found in the exclusive milk-fed calves as compared with the calves fed milk and supplement, especially during the latter period of the experiment.

### *Post-Mortem Findings*

The results of post-mortem examinations are given in table 2.

Differences in total body weight between the anemic and non-anemic calves preclude a direct comparison of the effect of iron and copper on the development of body organs. However, there was a marked and consistent difference in the size of the testes and spleen, those of the calves fed iron and copper being much larger.

The kidneys appeared normal in every respect.

The iron and copper content of the calf livers of Series II (Table 3) substantiate the work of others (8, 9) in demonstrating that the liver acts

TABLE 3  
*Iron and copper content of livers*

CALF NO.	WEIGHT GN.	IRON PCT	TOTAL IRON MG	COPPER PCT	TOTAL COPPER MG
LOI I					
M 406	2403	0.00366	87.9	0.00122	29.3
M 408	3809	0.00241	91.8	0.00087	33.1
M 414	3084	0.00308	94.9	0.00232	71.5
Av.	3099	0.00295	91.5	0.00144	34.6
LOI II					
M 407	5442	0.00715	389.1	0.00992	539.8
M 411	5669	0.00752	426.3	0.01347	763.6
M 416	2648	0.00992	262.7	0.01305	345.5
Av.	4586	0.00783	359.3	0.01198	549.6

as a storehouse for iron and copper. The iron values given compare favorably with those reported by Elvehjem and Peterson (7), but, according to the copper values reported by Lindow, Elvehjem, and Peterson (16), the calves fed iron and copper were receiving a considerable excess of copper.

### GENERAL DISCUSSION

The hemoglobin values given in table 1 indicate that the exclusive milk-fed calves (Series I), as compared with the calves fed milk, iron and copper were getting enough iron from some source to effect an increase in the hemoglobin content of their blood, especially during the last 3 months of the experiment. Considerable trouble was encountered throughout this series in keeping all the calves from eating shavings. At times they would tear

or wear holes in their muzzles, allowing them to eat wood shavings or lick the iron stalls. Towards the latter part of the experiment the calves fed whole milk exclusively (Lot I) required new muzzles about every 2 or 3 weeks. The calves in Series II were practically unable to eat any foreign material because of the new type of muzzle made from 0.040-inch pyrolinviscoloid. We feel that the use of a satisfactory muzzle in Series II was largely responsible for the more uniform results obtained.

The rate of growth of the first series, as compared with the second series, indicates that the system of feeding was inadequate in Series I in that it did not supply the calves with sufficient nutrients for rapid growth. The limiting factor may have been not only a shortage of total nutrients, as pointed out by Hughes and Cave (13), but an inadequate supply of vitamin D, as indicated by calcium and phosphorus determinations of the blood serum, and probably a deficiency of manganese, as indicated by Kemmerer, Elvehjem, Hart, and Fargo (14). The rate of growth of the calves fed iron and copper in Series II was, with one exception (M 416), very rapid throughout the experiment. M 416 grew very slowly for the first 4½ months but very fast during the remaining period of the experiment. The thyroid gland from this calf weighed 73 grams (2.7 times heavier than the average weight of thyroids taken from the other five calves in Series II), indicating iodine deficiency at birth.

No external symptoms of vitamin A deficiency were noticed in any of the calves, even though the vitamin A content of the livers from the last series of calves was about one-seventh the amount found in livers from normal calves of the same age.

#### SUMMARY

Six pairs of Holstein male calves were used in a study of the susceptibility of calves to nutritional anemia when fed whole milk exclusively and of the effect of supplementing such a diet with inorganic iron and copper.

Physical appearance, growth, determinations of the number of erythrocytes and amount of hemoglobin in the blood, and post-mortem examination showed that the calves on milk alone developed nutritional anemia while those receiving iron and copper in addition to milk did not.

Considerable difference in appearance and size of the internal organs was revealed on post-mortem examination. Of particular interest in this connection was the marked difference in size of the testes and spleens, those of the calves receiving the mineral supplements being much larger.

The livers of the calves fed iron and copper were found to contain large quantities of these two minerals as compared to the amounts found in the livers of calves fed milk exclusively.

## CONCLUSIONS

Holstein male calves develop nutritional anemia when fed whole milk exclusively.

The daily addition of 400 mg. or more of inorganic iron and 40 mg. or more of inorganic copper to an exclusive whole-milk diet will prevent the development of nutritional anemia in calves.

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## American Dairy Science Association Announcements

### *Housing Plans for the 1935 Meeting*

The School of Agriculture dormitories will be available to those attending the American Dairy Science meeting at University Farm, St. Paul. The rooms are large, airy and well lighted and it shall be the aim of the committee on housing to see that everyone is comfortable and happy while here, at a cost of 75 cents per person per night. The members of the committee on housing and registration are Superintendent J. O. Christianson and Dr. W. E. Peterson at University Farm.

If you wish to bring the family, there are several suites where accommodations may be arranged. However, it is very important that all reservations be made as early as possible. The postcards which the members returned to the Program Committee were intended only as a general guide. *Those who indicated that they expect to make reservations at the dormitories should not regard such indication as an actual reservation.* This must be made directly with the chairman of the housing committee. *Send reservations to Superintendent J. O. Christianson, University Farm, St. Paul, Minn.,* and state carefully the number of persons in your party, men, women and children.

### *Reduced Railroad Fares*

Reduced fares will be in effect from all points for this meeting, available as "Convention Fares" of a fare and one third for the round trip. Certain territories will have very low "Summer Tourist" fares, and "Summer Tourist Short Limit" tickets on sale, that will provide an opportunity to extend your trip, if desired. Consult your local railroad ticket agent for cheapest fares from your particular location, and he will advise you regarding cost and possible extensions of trip beyond St. Paul.

### *Program of Entertainment for the Ladies*

#### June 24—Monday

8:30 p. m.—Informal social get-together for members and their families, Home Economics Building, University Farm.

#### June 25—Tuesday. Hosts for the day—Twin City (St. Paul and Minneapolis) Dairy Industry.

9:00 a. m.—12:30 p. m. Sight-seeing automobile trip of the Twin Cities.

12:45 p. m.—Complimentary luncheon, Radisson Inn, Lake Minnetonka.

2:30 p. m.—Return to University Farm Campus.

- 4:00 p. m.—Members and their families leave for trip to Land-O-Lakes Creameries, Inc.
- 6:00 p. m.—Complimentary dinner and entertainment as guests of Land-O-Lakes Creameries, Inc.
- June 26—Wednesday. Host for the day—General Milk Inc.
- 9:00 a. m.—Busses will call for the ladies at University Farm and conduct them on a tour of the University of Minnesota Campus, Minneapolis, and of the Washburn Crosby Flour Mills.
- 12:00 noon—Complimentary luncheon at Nicollet Hotel, Minneapolis.
- 2:30 p. m.—Return by busses to University Farm, St. Paul.
- 4:00 p. m.—Tea, Fireplace Room, Home Economics Building, University Farm. Mrs. Walter C. Coffey, *Hostess*.
- 5:00 p. m.—Leave for Minneapolis Automobile Club.
- 7:00 p. m.—Subscription banquet and entertainment at Minneapolis Automobile Club.
- June 27—Thursday. Shop, golf or other optional activities; or merely rest.

### *Suggestions for Presentation of Papers*

The Society of American Bacteriologists and the American Chemical Society have recently published the following suggestions to their members for the purpose of increasing the effectiveness of their scientific programs. These suggestions are so clear cut and pointed that they are well worth passing on to the members of our association who may not have seen them.

“1. Arrangement of Material. Manuscripts as prepared for publication are seldom suitable for oral presentation. The paper should convey clearly to the hearer: (a) the purpose of the work; (b) the experimental method; (c) the results obtained; and (d) conclusions. The nature of the material and the time available for presentation will determine the degree of emphasis to be placed on each subdivision. The author should make certain by trial against his watch that the essential points can be adequately presented in the time allotted to the paper.”

“2. Statement of Purpose. Orient the audience clearly as to the nature and purpose of the work. A lengthy historical review is generally out of place.”

“3. Technic. Describe the experimental method employed, so as to indicate the principles involved. Omit details of apparatus or procedure unless there is some particularly novel development. Such data may belong in the published paper but will bore your audience.”

“4. Statement of Results. Present the results graphically, preferably with diagrams. Lantern slides are more clearly seen than hand-drawn charts. These slides should be of standard size (3.25 x 4 inches) and should project clearly on the screen. Regardless of who has made the charts or slides, try them from the point of view of the audience

before presenting them at the meeting. Do not read tables, a procedure which wastes time and destroys interest but point out the general trend of the data."

"5 Conclusions. Summarize the evidence and discuss the importance of the results or conclusions to the particular field of research involved."

"6 Manner of Presentation. Do not read from a manuscript verbatim. Talk directly to your audience in a clear loud voice. Do not face blackboard or screen while speaking. Articulate distinctly."

"7 Many exceptions to and modifications of the above suggestions will apply in particular instances. Nevertheless, general adherence to the points brought out will go far in eliminating the valid criticisms which have been aimed at our programs."

L. S. PALMER, *Chairman*  
Program Committee

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## THE EFFECT OF MASTITIS UPON MILK PRODUCTION

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It is generally accepted that each quarter of a cow's udder functions as a separate physiological unit. Parks (1) has given figures for the minimum, the maximum, and the differences in calcium content of milk from each quarter of the udder of five cows. Benton (2), after studying the subject of coagulation, presented values for pH and percentage of butterfat from different quarters of the same udder and concluded that each quarter of a cow's udder is a separate physiological unit. Mattick and Hallett (3) determined the yield, titratable acidity, hydrogen-ion concentration, time of rennet coagulation, total nitrogen, casein nitrogen, amount of butterfat, total solids, and ash content of the milk from different quarters of the same udder. They found wide differences in the results of these determinations in the milk produced by the front and rear quarters, and significant differences when a comparison was made between the secretion from the opposite front, or the opposite rear quarters.

Very little work has been done on the effect of disease on the composition of milk. Cranfield and Ling (4) analyzed milk from a cow which secreted abnormal milk for three lactations and found that the ratio of calcium to phosphorus varied from 0.78 to 1.65. Koestler (5) found that abnormalities in secretion were accompanied by changes in composition which follow a general rule. The amount of globulin, chlorine, sodium and sulphate were increased, while the lactose, potassium, magnesium, calcium, and phosphorus content were decreased.

Mastitis is very common in all parts of the world, and the most conservative estimates place the rate of infection of all cows of producing age at from 15 to 40 per cent. Steelman, according to Rosell (7), in an examination of 170 herds in Germany, found that 52 per cent of all the cows possessed diseased udders, while the workers at the Kiel Station in the same country report that 66 per cent of some 50,000 milk samples examined by them yearly gave evidence of the disease. Minnott (8) reported the examination for streptococci of 850 milking cows in sixteen herds in Great

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Britain He found that not less than 333 of these cows were infected. The incidence in each herd ran from 11 to 65 per cent. Hucker (9) reporting in this country found that 90 per cent of 221 cows examined, showed induration or fibrosis of the udder in one or more quarters. Forty-eight per cent of all quarters discharged streptococci in the milk, while 9 per cent showed pus pockets on post mortem.

During the past year a study of the efficiency of the various tests (brom thymol blue, chlorides, cell count, and catalase) for the detection of mastitis has been carried on at The Pennsylvania Station. During the progress of this work, cows were found which gave a positive reaction to the tests in one or more quarters of the udder for a period of time, and then later seemed to be free from any abnormal udder condition as indicated by at least three of the four tests employed. This raised the question as to the effect of such infections upon the efficiency of the secretion from the diseased quarters as compared with the secretion from the opposite healthy quarters.

Some work (6) has been reported which indicates that the rear quarters produce more milk than the fore quarters, and that a variation is often found between the amount of secretion in the fore quarters, and also between the production of the rear quarters.

In order to determine the extent of this variation, eighteen cows with normal udders were selected. For a period of four to six months these individuals gave a negative reaction to the four tests for mastitis. All tests, both for mastitis and butterfat, were run every two weeks. The cows were milked by hand into a bucket divided into halves so that the milking could be carried out in the normal manner. The milk from each quarter was weighed and the percentage of butterfat determined by the Babcock test. The average amount of milk, pounds of butterfat, and percentage of butterfat from each of the four quarters of the udders of these eighteen cows is given in table 1.

TABLE 1

*The average amount of milk produced from each of the four quarters of non-infected udders*

QUARTER	AVERAGE MILK PRODUCTION	AVERAGE BUTTERFAT PRODUCTION	AVERAGE BUTTERFAT
	<i>Lbs</i>	<i>Lbs</i>	<i>Per cent</i>
Right Front	2.9	.1056	3.64
Left Front	2.6	.0919	3.53
Right Rear	3.3	.1178	3.57
Left Rear	3.3	.1195	3.62

From these figures it is evident that only a small variation may exist between the amounts of milk secreted by the fore quarters and by the rear

quarters of non-infected udders. The average variation in the pounds of milk produced by opposite quarters ranged from zero for the rear quarters to ten per cent for the fore quarters. The average variation in pounds of butterfat ranged from one per cent between the opposite rear quarters to twelve per cent between the opposite fore quarters.

For the remainder of the study 86 cows were selected which showed an infection in one quarter of the udder as indicated by a positive reaction to at least three of the four tests employed, while the opposite quarter remained negative to all tests.

These quarters were milked by the same method employed on the non-infected udders, and the same data recorded. A considerably greater difference was found to exist between the production of the non-infected quarters and the opposite infected quarters than in the trial where both quarters were normal.

The data in table 2 show that the non-infected fore quarters produced 0.8 of a pound or 34.8 per cent more milk, and 0.0379 of a pound or 34.3

TABLE 2  
*Comparison of production from opposite non-infected and infected fore quarters*

QUARTER	NUMBER	AVERAGE MILK PRODUCTION	AVERAGE BUTTERFAT PRODUCTION	AVERAGE BUTTERFAT
Non-infected	41	Lbs. 2.3	Lbs. .1102	Per cent 4.79
Infected	41	1.5	.0723	4.82

per cent more butterfat than did the opposite infected quarters. If the variation between the milk production of normal quarters as indicated in table 1 is taken into consideration, then there is a difference of approximately 25 per cent more milk produced by non-infected than by infected fore quarters. In the case of butterfat the difference is around 21 per cent in favor of the non-infected quarters.

The non-infected rear quarters as indicated in table 3 produced 1.2 pounds or 31.6 per cent more milk and 0.058 of a pound or 40 per cent more butterfat than the opposite infected rear quarters. There was practi-

TABLE 3  
*Comparison of production from opposite non-infected and infected rear quarters*

QUARTER	NUMBER	AVERAGE MILK PRODUCTION	AVERAGE BUTTERFAT PRODUCTION	AVERAGE BUTTERFAT
Non-infected	45	Lbs. 3.8	Lbs. .1448	Per cent 3.81
Infected	45	2.6	.0868	3.34

cally no difference in the production of milk or butterfat between the non-infected rear quarters, as indicated in table 1, therefore the non-infected rear quarters are approximately 31.6 per cent more efficient in milk production and 40 per cent more efficient in butterfat production than the infected rear quarters.

A comparison of the production of all the opposite non-infected and infected quarters is given in table 4. It will be noted that the non-infected

TABLE 4

QUARTER	NUMBER	AVERAGE MILK PRODUCTION	AVERAGE BUTTER-FAT PRODUCTION	AVERAGE BUTTERFAT
		<i>Lbs.</i>	<i>Lbs.</i>	<i>Per cent</i>
Non-infected	86	3.1	.1280	4.13
Infected	86	2.1	.0798	3.80

quarters produced on the average one pound of milk and 0.0482 of a pound of butterfat more than the infected quarters. This is equivalent to 32.3 per cent more milk and 37.6 per cent more butterfat from the non-infected quarters than was produced by the infected quarters.

A variation in milk and butterfat production was found to exist between the opposite fore quarters and the opposite rear quarters of non-infected udders. These figures are given in table 1.

After allowing for the maximum variation in milk and butterfat production found between non-infected quarters, it would appear that the non-infected quarters are about 22 per cent more efficient in producing milk and 24 per cent more efficient in butterfat production than the mastitis infected quarters.

#### CONCLUSIONS

1. Only a small variation exists between the pounds of milk, per cent butterfat, and pounds of butterfat produced by opposite non-infected quarters of a cow's udder.

2. A considerable variation may be found between the production of opposite mastitis infected and non-infected quarters of a cow's udder.

3. Mastitis infection apparently reduced milk production approximately 22 per cent and butterfat production 24 per cent after allowing for the maximum variation found in the milk and butterfat production of non-infected quarters.

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# THE HYDROGEN-ION CONCENTRATION OF CREAMERY WATERS AND THEIR RELATIONSHIP TO WASHING BUTTER\*

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## INTRODUCTION

The washing of butter in the grain, after churning and before working, has been an accepted practice for many years; it being considered good practice to wash the butter twice, in such a manner that the last wash water leaves the churn free from buttermilk. However, excessive washing of butter tends to produce a flat flavor (5), but is recommended for poor quality butter.

The biological purity of the water used for washing has received considerable attention (4, 9, 13). Poor keeping quality and actual defects in the flavor of the butter at times have been attributed to infection of butter with undesirable organisms from the wash water (13, 14).

The mineral constituents of the creamery water have received their chief attention insofar as they affect the boiler and the creamery equipment that has to be washed (2). On the other hand, the reaction of the water used for washing butter has not received much consideration.

Knowing that several creamery supplies are alkaline in reaction, about pH 7.6, it was thought that the texture of butter under certain conditions might be affected by washing with such water. It is known that several proteins, *e.g.*, casein, are more soluble in an alkaline reaction than an acid reaction; therefore, were a protective layer removed from the grains of butter by washing with alkaline water more free fat would be liberated (19). Such a theory might possibly answer some of the unknown causes of short grained and sticky butter (19). On the other hand, certain proteins, such as casein, form salts with alkalis and alkaline earths under alkaline conditions, which might be adsorbed on the grains of butter and possibly cause texture defects. Where the texture of butter is found to be adversely affected by alkali wash waters, it would be a relatively simple matter to standardize the water to a most desirable pH, using hydrochloric or some other harmless acid.

Therefore, it is the object of this work to determine the prevalence of alkaline water in Eastern Washington and Idaho and what effect the reaction of such water has on butter which is washed with it.

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## LITERATURE

The early work of this station by C. W. McCurdy (7, 8) shows that water in Idaho, particularly in the dry belts and the irrigated areas, is likely to be alkaline in reaction.

Wright (17), in an extensive study of surface and subsurface waters of the Yakima and Klamath reclamation projects, gives the pH of the water from 79 domestic wells, which appears in table 1, and shows that all these waters are more or less alkaline in reaction, about one-quarter being over pH 8.0.

TABLE 1

*The reaction of waters from domestic wells in the Yakima and Klamath project (17)*

NUMBER OF WELLS		
Ranging from pH 7.0 to 7.4	Ranging from pH 7.5 to 7.9	Ranging from pH 8.0 and above
46	15	18

Larsen *et al.* (6), washing butter with alkaline wash water, found that unsalted butter deteriorated one point in flavor more than the control. With salted butter no difference in flavor was noted. Their work does not exclude deterioration that was of biological origin.

Brown (1) conducted experiments on the curd content of butter and showed that the larger the grains of butter in the buttermilk before washing the greater was the curd content of the resulting butter. Whether the removal of the curd was entirely physical or partly chemical was not shown.

Several authorities (12, 15) show that the substance adhering to or adsorbed on the fat globules is of a protein-like nature. The examination of the nature of the protein surrounding the fat globules in milk by Titus *et al.* (15) lead them to conclude that it is very closely related to, if not identical with, casein. On the other hand, Palmer and Wiese (12) find that the material, most closely adsorbed on the surface of the fat globules in cows' milk, is composed of a mixture of protein and phospholipides. This protein's physical properties, percentage of nitrogen, sulfur, and phosphorus do not correspond with those of any other milk protein. It constitutes the major part of the so-called fat globule membrane. It is unnecessary to go further into the discussion of this still debatable question but rather to accept the general conclusion, that they and several other investigators find, that the substance surrounding the fat globules is largely of a protein-like nature. Since milk when treated with ether does not give up its fat, or only to a slight extent, until a small amount of acid or alkali

has been added to dissolve this protein (15) it would seem that the reaction of the wash water might be of significance in the manufacture of butter.

#### MATERIALS AND METHODS

*Natural Waters:* With the exception of the pH determination made on the various creamery water supplies, the water used in these experiments was the supply of the Washington State College at Pullman, which at all times during the investigation showed a pH of 7.6. Its reported analysis by the Cochrane Corporation, Philadelphia, Pennsylvania, is given in table 2.

TABLE 2  
*Analysis of water supply at Washington State College*

	PARTS PER MILLION	EQUIVALENT PER MILLION
Calcium (Ca)	24.2	1.21
Magnesium (Mg)	12.4	1.01
Sodium (Na)	30.8	1.34
Iron oxide of alumina ( $R_2O_3$ )	4.0	0.22
Silica ( $SiO_2$ )	48.0	1.59
Bicarbonate ( $HCO_3$ )	207.0	3.39
Carbonate ( $CO_3$ )		
Sulphate ( $SO_4$ )	8.0	.17
Chloride ( $Cl_2$ )	Trace	
Volatile & organic	10.0	
Total solids in solution	240.0	
Suspended matter	Trace	
$CO_2$ Free	14.4	0.65
Hardness in terms of $CaCO_3$	111.0	2.22
Free Acid, pH	7.8	

#### *Hypothetical combinations*

	GRS./GAL.
$CaCO_3$	3.56
$MgCO_3$	2.51
$SiO_2$	2.80
Iron and oxide of alumina ( $R_2O_3$ )	0.23
Sodium carbonate ( $Na_2CO_3$ )	3.62
$Na_2SO_4$	0.70
$NaCl$	Trace
Volatile of organic	0.58
Total solids in solution	14.00
Suspended matter	Trace
$CO_2$	0.82

The buffer curve for the Washington State College water supply is given in figure I, which shows that considerable acid must be added to the water before pH 6.6 is reached, which is about the reaction of sweet cream.

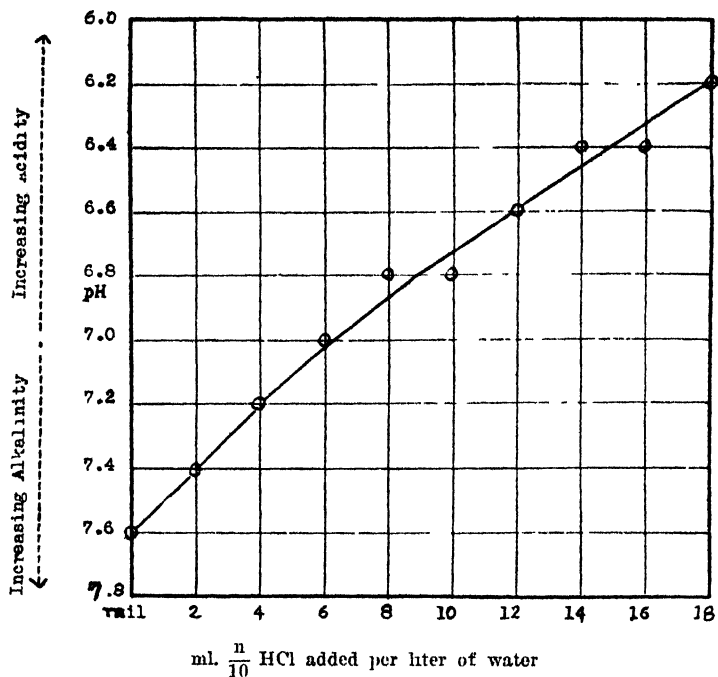


FIG. I. THE BUFFER VALUE OF THE NATURAL ALKALINE WATER SUPPLY.  
TEMP. 59° F.

*Distilled Water:* The distilled water when used for washing the butter was double distilled but showed a pH of about 6.6 due to dissolved  $\text{CO}_2$ . Both waters were stored in Pyrex glass. Neither water contained any measurable quantity of nitrogen when determined by the Kjeldahl method. In spite of this, separate duplicate blank determinations were run with each experiment.

*Acids:* Where required the natural water was acidified with C.P. lactic acid in the commercial trials and redistilled N/10 hydrochloric acid when washing the small lots of butter.

*Cream:* The sweet cream was obtained from the University of Idaho herd of Jersey and Holstein cows and was pasteurized before separation.

The commercial cream was sour gathered cream, from two local creameries, neutralized and pasteurized in coil vats in the creamery.

*pH Measurements of Water:* All pH measurements of water were done by colorimetric methods against known buffer standards and checked with several indicators, which were prepared by the method given in the Handbook of Chemistry and Physics (3). A further precaution was taken to

standardize the indicators electrometrically to the pH of the mid-point of their color change. The determinations were made at about the temperature of washing butter as it was considered this gave a better index of the conditions to which the butter was subjected during washing.

*pH Measurements of Cream and Butter:* The quinhydrone method, using a gold electrode and a Leeds Northrup No. 7654 pH indicator, was used to determine the pH of the cream and butter serum. The readings were made at 25° C. The butter serum was prepared by centrifuging according to the method of Weckel (16).

*Method of Washing the Butter:* The commercial churnings, made in a small single roller Cherry churn, were washed with the Washington State College water, tempered and acidified with lactic acid as required. Four buckets of water were used in place of spraying the butter and then a first and second wash water in the usual commercial proportion of about one of butter to two of wash water.

The churnings, to determine the solubility of protein in the various wash waters, were done in a 12-gallon Superior Sanitary crock churn. The 300 grams of butter required in each case were carefully weighed into a 2-litre beaker which contained 600 ml. of water at the required pH. Each series was uniformly stirred, held for 2 hours in a cool room, and the wash water then filtered off from the butter through 4 thicknesses of cheese-cloth, previously washed in distilled water and dried. The wash water was then analyzed for nitrogen according to the following method: (Note: Though a two-hour washing period for butter is not commercial practice, Whittier (18) has shown that equilibrium is attained in this time with milk.)

*Nitrogen determination:* The nitrogen (protein) was determined in the wash water by the Kjeldahl-Gunning-Arnold method (11), a suitable known volume of wash water being used according to whether or not the butter had been previously washed with distilled water. After subtracting the blank determinations the results are expressed in milligrams of nitrogen per litre of wash water. As an additional precaution, ammonia was driven off from the wash water in the Kjeldahl flask before the sulphuric acid was added.

#### EXPERIMENTAL

*The Reaction of Creamery Water Supplies:* Through the cooperation of R. L. Nelson and Mr. D. L. Fourn, the pH of a number of creamery waters were determined in the creameries where the butter was washed. The results of these determinations are given in table 3 which shows:

1. A similar distribution to that in table 1.
2. All the waters were more alkaline than the buttermilk made from sweet cream.

TABLE 3  
*The reaction of waters from creamery supplies Sept., 1933, to March, 1934*

LOCATION	NUMBER OF SUPPLIES			
	Ranging from pH 6.8 to 6.9	Ranging from pH 7.0 to 7.4	Ranging from pH 7.5 to 7.9	Ranging from pH 8.0 and above
South East Washington (De- termination by R. L. Nelson)	5	5	6	1
South Idaho (Determina- tion by D. L. Fourt)		4	4	
Eastern Washington and Western Idaho	1	1	3	

3. The difference may be considered significant in about half the cases which are pH 7.5 or above.

*The Change in the Reaction of Creamery Water When Used for Washing Butter:* Table 4 gives the results from a set of commercial churnings, churned to grains a little bigger than wheat and washed according to the method described. Table 4 shows

1. That the natural alkaline waters at pH 7.6 (churnings 1, 3, 6, and 9) were affected in reaction by the first washing of the butter. However, in the second washing the water flowed from the butter practically at the same reaction as when added. Note: The City of Spokane water gave similar results.

2. The waters neutralized to pH 7.0 by acid (churnings 4, 7, and 10) were rendered acid by the first washing of the butter but were unchanged in the second washing.

3. The water adjusted to the reaction of the cream (churnings 2, 5, 8, and 11) remained at practically the same reaction throughout washing.

4. The pH of the serum of the butter, though slightly less acid, when washed with alkaline water was not significantly changed. The greatest difference was between churnings one and two, 0.15 pH.

5. In general it would appear that the surfaces of the grains of butter were affected by the wash water rather than the mass, as the pH of the serum of the butter was unchanged.

*The Change in the Reaction of Wash Water When Used for Washing Butter:* A further series of experiments were conducted on the change in the reaction of wash water, using (a) the natural alkaline wash water, (b) the same acidified to the pH of the cream and (c) water acidified to about the isoelectric point of casein. The washing of the butter was done in 2-litre beakers as previously described. Instead of washing the same butter twice with the prepared waters, distilled water was used so as to remove

TABLE 4  
Change in the reaction of water after being used for washing butter

WATER SUPPLY	DATE	CHURN- ING NO.	CREAM		pH OF WATER SUPPLY	* ACIDIFIED TO pH	pH OF WASH WATER WHEN RUN OFF FROM THE CHURN		SERUM OF FINISHED BUTTER pH	REMARKS
			Acidity %	pH			1st	2nd washings		
City of Spokane	Dec. 23 1933		.23		7.6	not changed	6.8	7.4		Large Dual Churn
		1	.23	5.95	7.6	not changed	7.4	7.6	6.2	
		2	.225	5.95	7.6	6.0	6.1	6.1	6.05	
		3	.215	6.35	7.6	not changed	7.0	7.4	6.65	
		4	.215	6.35	7.6	7.0	6.4	7.0	6.65	
Washington State College	Dec. 30 1933	5	.215	6.35	7.6	6.35	6.4	6.35	6.60	Butter made and washed in the small Cherry Churn
		6	.26	6.15	7.6	not changed	6.6	7.5	6.25	
		7	.25	6.15	7.6	7.0	6.4	7.0	6.30	
		8	.25	6.25	7.6	6.3	6.3	6.3	6.30	
		9	.20	6.5	7.6	not changed	7.3	7.6	6.90	
	Jan. 2 1934	10	.20	6.5	7.6	7.0	6.9	7.0	7.0	
		11	.20	6.55	7.6	6.5	6.5	6.6	7.0	

\* With Lactic Acid.

TABLE 5  
Change in the reaction of water when held in contact with butter in the granular form

Type	CREAM	WATER			UNWASHED BUTTER HELD IN CONTACT WITH WATER		BITTER PREVIOUSLY WASHED ONCE WITH DISTILLED WATER HELD IN CONTACT WITH WATER		BITTER PREVIOUSLY WASHED TWICE WITH DISTILLED WATER HELD IN CONTACT WITH WATER	
		pH	pH	Acidified by N/10 HCl pH	1 hour pH of water	2 hours pH of water	1 hour pH of water	2 hours pH of water	1 hour pH of water	2 hours pH of water
Sweet Pasteur- ized	Acidity %	pH								
	.125	6.65	7.6	not changed 6.6 4.7					7.6 6.7 5.2	7.6 7.0 5.8
" " "	.12	6.65	7.6	not changed 6.6 4.6			7.6 6.6 5.0	7.6 6.8 5.3	7.6 6.8 5.2	7.6 7.0 5.2
" " "	.125	6.65	7.6	not changed 6.7 4.7			7.6 6.7 5.2	7.6 6.8 5.2	7.6 6.7 5.2	7.6 6.9 5.2
" " "	.125	6.65	7.6	not changed 6.7 4.7			7.6 6.7 5.2	7.6 6.7 5.2	7.6 6.7 5.0	7.6 6.9 5.4
Sour Cream Neutralized and Pasteur- ized	.285	6.0	7.6	not changed 6.0 4.7	6.4 6.1 5.0	6.4 6.0 5.2	7.3 5.0	7.2 6.2 5.2	7.4 6.2 5.4	7.4 6.3 5.4
	.33	6.2	7.6	not changed 6.2 4.7	6.8 6.2 5.8	6.8 6.2 5.8	7.5 6.4 5.2	7.5 6.4 5.3	7.5 6.4 5.0	7.6 6.5 5.0
	.33	6.25	7.6	not changed 6.3 4.7	6.6 6.2 5.2	6.5 6.2 5.8	7.4 6.3 5.0	7.4 6.4 5.2	7.6 6.4 5.0	7.4 6.5 5.0

mechanically, as far as possible, the buttermilk before the acid or alkaline action of the water affected the butter grains.

Table 5, in addition to confirming the statements one and three, in the previous experiment, shows that:

1. In most cases after the wash water was held in contact with the grain of the butter for one hour, the pH of the water remained practically constant till the end of the second hour. Note: In two cases where the period was continued for twenty-four hours the change was still slight.

2. Being more acid, butter made from sour neutralized pasteurized cream affected the change in reaction of the wash water more than sweet pasteurized cream.

3. The acid wash water at pH 4.7 changed most in reaction towards the alkaline side in the second and third washings; at least due in part to its being only very slightly buffered between pH 4.7 and 5.6.

4. The mechanical washing by distilled water removes the free buttermilk, which was the main cause of the change in reaction of wash water towards the pH of the cream.

*The Removal of Nitrogen (Protein) from Butter in the Grain by Washing in Water at Various Hydrogen-ion Concentrations:* Churnings were made in a 12-gallon crock churn and 300 grams of the butter in the grain weighed out into 2-litre beakers containing 600 ml. of water at the required pH. The 3 beakers, containing butter and water in each section, i.e., unwashed butter, washed once with distilled water, or washed twice with distilled water, were treated as far as possible identically except for the difference of pH of the final wash water. After holding in contact with the butter for 2 hours, the filtered wash water was analyzed for nitrogen. The results, the average of duplicates, are expressed in milligrams of nitrogen per litre.

Before discussing table 6, it is as well to point out that the method used of weighing out 300 grams of butter in the grain, even though fairly firm and well rounded, leaves considerable chance for carrying uneven quantities of buttermilk, or water used in the previous washing, into the next wash water. This point most probably accounts for the uneven results in table 6, which only can be considered as showing certain trends, namely:

1. On an average, with the sweet cream butter in the grain, more nitrogen (protein) was removed in the alkaline wash water, less in that which was the same pH as the cream and still less in the acid water, indicating that the alkaline water removed more protein from the butter. Three individual exceptions occur, two in experiment 2, and one in experiment 3.

TABLE 6  
Removal of nitrogen (protein) from butter in the grain by wash waters at various pHs

EXPERIMENT	CREAM			UNWASHED BUTTER				WASHED ONCE WITH DISTILLED WATER				WASHED TWICE WITH DISTILLED WATER					
	Type	Acidity %	pH	Nitro- gen per litre of water after washing mg.	Nitro- gen per litre of water after washing mg.	Nitro- gen per litre of water after washing mg.	Water for wash- ing pH	Nitro- gen per litre of water after washing mg.	*Water for wash- ing pH	Nitro- gen per litre of water after washing mg.	Water for wash- ing pH	Nitro- gen per litre of water after washing mg.	*Water for wash- ing pH	Nitro- gen per litre of water after washing mg.	*Water for wash- ing pH	Nitro- gen per litre of water after washing mg.	
1 2 3 4 Average for sweet cream	Sweet Pasteurized	.125	6.65														
	"	.120	6.65														
	"	.125	6.65														
	"	.125	6.65														
	Average for sweet cream	.124	6.65														
5 6 7 8 Average for Sour Neutralized and Pasteurized Cream	Sour Cream Neutralized and Pasteur- ized	.285	6.0														
	"	.29	6.3	142.9	135.9		7.6	13.2	6.0	12.1	4.7	11.1	4.8	6.0	4.1	4.7	3.6
	"	.33	6.2	87.8	78.4	93.3	7.6	9.5	6.2	9.5	4.7	8.1	7.6	6.3	2.7	4.7	2.1
	"	.33	6.25	107.4	112.3		7.6	8.7	6.3	10.0	4.7	10.1	7.6	6.3	3.5	4.7	2.5
	Average for Sour Neutralized and Pasteurized Cream	.31	6.2	112.7	99.3	10.5				10.5		9.8	4.0		3.4	4.7	2.8

\* Acidified with n/10 HCl.

2. With the sour neutralized cream butter, the removal of nitrogen (protein) by the wash water gave too irregular results to draw conclusions, except where the grains had been twice washed with distilled water. Here the general trend was the same, namely: as the acidity increased, the nitrogen (protein) removed diminished.

3. The lack of uniformity in table 6 calls for further investigations by other methods before it can be said definitely that alkaline wash water removes more nitrogen (protein) from the surface of the butter than acid wash waters.

4. Though not intended as a part of this investigation it was noted that the nitrogen in the second and third waters showed a very marked difference when comparing sweet and neutralized cream; the proportion being about four times as great in the former as the latter. As the treatment had been similar in both cases, the problem might form an interesting study.

#### DISCUSSION OF RESULT

The presence of water of an alkaline reaction in Idaho and Washington has previously been reported (7, 8, 17). The results of our tests show that such water is frequently used as a creamery supply for washing butter in the grain. The analysis and buffer value of one such water supply (the Washington State College, Pullman) is given in detail and this supply was used for remainder of the experimental work in washing butter in the grain.

The first experiments to determine what happened to the reaction of the butter and the alkaline wash water showed that the water became more acid due to free buttermilk (i.e., toward the p<sub>H</sub> of the cream) in the first washing of the butter. While in the second washing the reaction of the water remained almost unchanged.

The reaction of the serum of the resulting butter washed twice was not significantly changed by the reaction of the wash water whether or not the wash water used was the same pH as that of the cream or alkaline. Therefore, whatever the effect of alkaline wash water may be, it is a surface effect.

A preliminary study made to determine the effect on the surface of the grains of butter by the change in reaction of the wash water showed a trend for the alkaline water to remove more nitrogen (protein). This removal was greater in proportion after most of the free buttermilk was removed. Owing to the lack of uniformity in results due to an error in the method which has no means of measuring the free water weighed out with the butter, this trend cannot be made a definite conclusion.

The problem is one which warrants further study as there are many texture defects of butter such as greasy and crumbly butter the causes of which are not answered fully. Wode (19) states, "The greasiness of butter

345 may be attributed to a too large amount of free fat and the crumbliness at a low temperature to an abundant crystallization of the free fat." If this statement is accepted as a general principle, the breakdown or removal of surface layers would be expected to have a marked bearing on texture defects by increasing the free fat in the butter.

The recommended practice of adding acid to a churn to prevent butter sticking during churning and working (5, 10) may in some ways be associated with alkaline waters. Further, with the introduction of metal churns the reaction of the wash water may play a more significant part. Also as the zeolite process is being recommended for dairies (2) and becoming more common; therefore, an alkaline soda wash water may require to be acidified before it gives the best results for washing butter.

Finally, if there were an advantage to be obtained by standardizing the pH of the wash water, the process could be simply, accurately, and satisfactorily done in most creameries.

#### SUMMARY

The reaction (pH) of a number of creamery water supplies in Idaho and Washington have been made at their respective creameries by colorimetric methods. As a considerable proportion of these waters were found to be alkaline in reaction, one such supply was employed to determine the effect of an alkaline water, when used for washing butter.

The first wash water, after washing the butter, was found to have a reaction between that of the water and cream while the second wash water retained the reaction of the original water. On determining the reaction of the serum of the butter, only slight changes were found; therefore, it was concluded that the changes in the reaction of the second wash water were largely a surface effect on the butter.

The nitrogen (protein) removed from butter in the grain by wash waters at various reactions was determined and found to be slightly greater in the alkaline wash waters as compared with those which had been acidified. After extraneous buttermilk was removed, which affected the reaction of the first wash water, the evidence of dissolved protein in the alkaline wash water was more marked.

The effect of alkaline water on the texture of the butter by possibly liberating more free fat in the butter was anticipated, but further experiments are necessary to establish the theory.

#### ACKNOWLEDGEMENTS

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## THE BACTERIOLOGY OF SWISS CHEESE. II. BACTERIOLOGY OF THE CHEESE IN THE PRESS

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The growth and activity of bacteria in the kettle contents during manufacture of Swiss cheese have been discussed in a previous paper (5). It has been shown that of the starter bacteria added to the milk in the kettle, only *Streptococcus thermophilus* grows to any extent in the kettle contents and that it causes little or no change in the acidity of the curd and whey. This paper will deal with studies on the cheese from the time the curd is dipped into the hoop until the cheese has been in the press for 21 hours.

Other workers have noted the presence of streptococci as well as lactobacilli in the cheese in the press. Orla-Jensen (7) observed that streptococci were active during the first hours the cheese was in the press and that the lactobacilli became active later. Thöni (9) found that cocci usually predominate in one day old cheese, but after a short time decrease rapidly, while the lactic rods increase and then make up 80 to 100 per cent of the flora; in small cheeses the lactic rods were found to predominate, even in the fresh mass. Most reported work on bacteria present in cheese deals with later stages of ripening. The growth of *S. thermophilus* and *L. helveticus* Bergey (1934) or *L. casei* Bergey (1923) in the cheese on the press has been referred to in an earlier publication (4) and the resulting development of acidity has been demonstrated.

### METHODS

Samples of the curd were taken by means of a No. 8 cheese trier and only that part of the plug four or more inches from the rind of the cheese was used for either chemical or bacteriological examination except in special experiments. Tests had shown that it was necessary to take samples this far from the rind because the outside of the cheese cools more rapidly, develops acidity at a greater rate, and has a much higher bacterial count than the inside. The one gram sample of cheese curd for bacteriological examination was carefully weighed and was ground with sodium citrate by the method described by Burkey (1): the cheese was placed in a 2½ inch mortar which had been previously steamed and then cooled to about 50° C. To the cheese in the mortar a few cubic centimeters of water at 50° C. were added from a sterile 9½ cc. water blank. The cheese and water were ground together for one minute; 0.2 gram of sodium citrate was added;

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the mixture was ground until a smooth homogeneous paste was formed; and finally the remainder of the water of the 9½ cc. blank was added. One cubic centimeter of this mixture contained one-tenth of a gram of cheese and was used in making further dilutions for microscopical and cultural examinations. When slides were to be made, dilutions were made in alkaline 50% alcohol and the preparations were fixed and stained by Burke's modification of the Gram method, as described in an earlier paper (5). For plate counts of bacteria, tomato milk-powder agar was used; citric acid agar was used for counts of aerogenes bacteria, and deep cultures in yeast extract-peptone-sodium lactate agar were used to determine numbers of lactate-fermenting bacteria.

Both types of starter bacteria, the streptococci and the lactobacilli, form chains when they increase in numbers. Consequently, an increase in numbers would be detected by the microscopic method, by which individual cells can be counted, better than by the plate method, which is based on counts of chains or clumps of bacteria. It is true that living and dead cells cannot be clearly distinguished by the usual microscopic method. The counts, however, were made from preparations stained by the Gram method and only the Gram-positive cells were counted. Cells old enough to be Gram-negative were considered dead. Any marked increase in numbers as determined by this method must be considered significant, while a small decrease may mean nothing more than the aging of some of the cells. A comparison of counts by the plate method and the direct microscopic method, is shown in Table 3.

In Table 1 a few counts are given of numbers of living bacteria determined by the method of Frazier and Boyer (3). Most of the counts reported in this paper had been made before this method was developed. The counts in Table 1 permit, however, a comparison of results by the new and by the usual Gram method and given an idea of how near counts of Gram positive organisms are to counts of living organisms.

#### RESULTS

Most of the data here reported were obtained from small experimental cheeses of about 50 to 55 pounds, green weight, made at Washington, D. C. Results with full-sized cheeses made at the Grove City Creamery, Grove City, Pa., will be included in the following paper.

A number of studies were made in which samples were taken every hour or every two hours from the time of dipping through the eighth or tenth hour and again at the twenty-first hour. Results indicated that samples at 0, 3, 8, and 21 hours gave a good indication of the growth of the starter bacteria in the cheese; therefore from most cheese only three or four samples were taken. Several workers have shown that bacteria grow

in colonies in the cheese curd. Hence the bacterial count on a gram of cheese may vary somewhat, depending upon the number and size of the colonies in the sample. For this reason the average of results from a number of cheeses is used in Figures 1 and 2. Examples of counts on individual cheeses are shown in Tables 1, 2, and 3, and it will be noted that occasionally a count does not fit well into the general trend of bacterial growth.

TABLE 1  
*Numbers of L. helveticus (39a) and S. thermophilus (C<sub>3</sub>) per gram in a cheese in the press*

TIME	PH	NOS. OF ORGANISMS - GRAM STAIN		NOS. OF LIVING ORGANISMS	
		39a	C <sub>3</sub>	39a	C <sub>3</sub>
HRS.					
0	6.40	3,000,000	48,000,000	970,000	35,100,000
1	6.28	3,480,000	36,600,000	1,850,000	15,000,000
3	5.94	1,500,000	77,100,000	2,660,000	64,000,000
7	5.35	630,000	231,000,000	2,500,000	190,000,000
9	5.35	630,000	248,000,000	2,080,000	184,000,000
11	5.26	11,000,000	248,000,000	11,600,000	187,000,000
13	5.22	182,000,000	257,000,000	184,000,000	181,000,000
22	5.06	193,000,000	349,000,000	116,000,000	226,000,000

TABLE 2  
*Numbers of L. bulgaricus (Ga) and S. thermophilus (C<sub>3</sub>) per gram in a cheese in the press*

TIME	TEMP. OF CHEESE	PH	NUMBERS OF ORGANISMS	
			Ga	C <sub>3</sub>
HRS.	°C.			
0	50.7	6.41	1,360,000	116,000,000
1	50.1	6.24	1,360,000	101,000,000
3	47.8	5.84	198,000	105,000,000
5	44.5	5.79	1,180,000	83,300,000
7	42.1	5.59	161,000,000	142,000,000
9	39.8	5.38	787,000,000	246,000,000

#### STARTER BACTERIA

Figure 1 shows the development of acidity as indicated by changes in pH and the growth of the two starter bacteria, *S. thermophilus*, C<sub>3</sub> strain, and *L. helveticus* Bergey, 1934, (*L. casei* Bergey, 1923) 39a strain. The data used in plotting the curves up to 9 hours were the average figures from a number of experiments.

Usually *S. thermophilus* decreased slightly during the first two or three hours that the cheese was in the press and then increased at a fairly rapid rate until about the seventh hour. After this the streptococci in-

TABLE 3

Numbers of starter bacteria and of *Aerobacter aerogenes* per gram in a cheese and a comparison of counts by the plate and direct microscopic methods

TIME	pH	DIRECT MICROSCOPIC COUNTS		PLATE COUNTS		
		39a	C <sub>1</sub>	Gas orgs. <sup>1</sup>	39a <sup>2</sup>	C <sub>1</sub> <sup>2</sup>
Hrs. Before dip		1,350,000	15,800,000	36,500,000	650,000	4,700,000
3	5.88		69,300,000	7,000	< 10,000	13,300,000
5	5.64		77,000,000	< 1,000	< 10,000	17,500,000
9 . . .	5.40	4,720,000	292,000,000		200,000	117,000,000
11	5.37	28,000,000	323,000,000		7,300,000	97,000,000
21 ..	5.10	161,000,000	153,000,000		213,000,000	31,000,000

<sup>1</sup> Counts on citrate agar.

<sup>2</sup> Counts on tomato milk-powder agar.

creased or decreased in numbers slowly and were sometimes slightly higher and sometimes considerably lower in numbers by the time the cheese was 21 hours old (Figure 1). In some experiments the numbers of streptococci were constant for the first three or four hours and in some increased slowly during that time. In most cases there was a rapid increase in numbers thereafter until the sixth to eighth hour. In some cheeses there was a decrease in the numbers of *S. thermophilus* after this first maximum, followed by an increase to a second and greater maximum. This often occurred when a weak lactobacillus culture had been used. In other cheeses

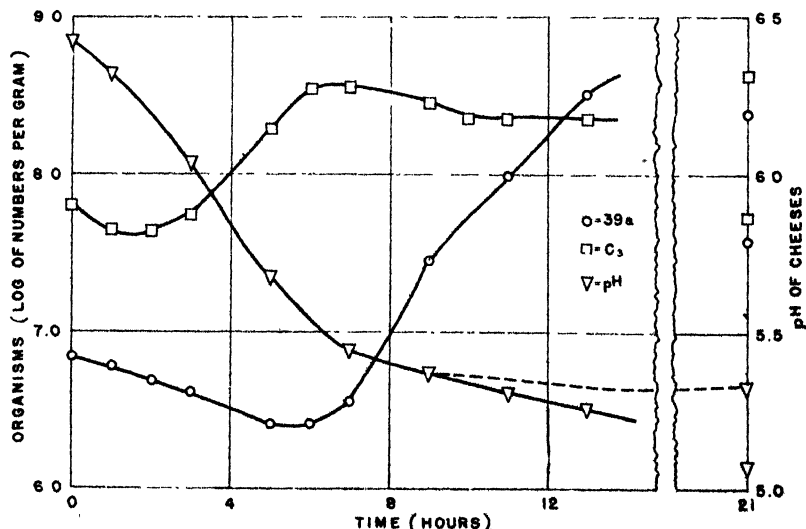


FIG. 1. GROWTH OF *L. helveticus* (39a) AND *S. thermophilus* (C<sub>1</sub>) IN SWISS CHEESE IN THE PRESS. AVERAGE RESULTS FROM A NUMBER OF CHEESES.

the numbers of *S. thermophilus* decreased gradually and there were comparatively few in the cheese at 21 hours from dipping time. This usually happened when an exceptionally active lactobacillus culture had been used.

As indicated in Figure 1, *L. helveticus* (39a) usually decreased slowly in numbers until about the sixth or seventh hour in the press, after which a fairly rapid increase took place. Individual cheeses often showed considerable variations from the results indicated in Figure 1. The variation which may occur in the curve of the development of the starter cultures and the pH is indicated in Figures 1 and 2 by duplicate symbols. Although 39a started to increase in numbers at about the sixth to eighth hour in the press when a strong starter culture was used, it usually did not start until several hours later when a weak culture was used. In the experiment shown in Table 1, 39a did not start to grow until about the tenth hour and in other cases growth did not start until the eleventh or thirteenth hour. The number of 39a organisms after 21 hours varied considerably in different cheeses. In some cheeses the lactobacilli apparently did not get well started and were low in numbers after 21 hours, while in other cheeses large numbers of 39a organisms were present.

Average figures show a fairly rapid drop in pH of the cheese during the first 7 hours in the press and a more gradual decrease thereafter. Most of this rapid decrease in pH takes place while only *S. thermophilus* ( $C_3$ ) is present in considerable numbers and is growing. Yet during the first three hours in the press the average number of  $C_3$  organisms present is only from 43,600,000 to 63,400,000 per gram. This number of organisms seems inadequate to cause the rapid drop in pH which is taking place. The increased size of these organisms, as measured under the microscope, may be an explanation of this unusual activity. Most of the cocci are three to four times as great in diameter and therefore 27 to 64 times as great in volume as cells in a culture grown at ordinary temperatures. In experiments with *E. coli*, Walker and Winslow (10) found an enormous increase in the rate of metabolic activity per cell toward the end of the initial lag period. The activity at this time was greater than that during the period of stable population and that during the phase of logarithmic increase. Later work (11) indicated that the increase in activity per cell could be accounted for partly, but not entirely, by the increase in the size of the cells late in the lag period, and the decrease in metabolic activity per cell during the logarithmic growth phase could be partially explained by a gradual decrease in cell size. Hansen (6) found that the fermentative ability of a thermophilic lactic organism at 55° was 30 times greater than that of *S. lactis* at 20° C. A further explanation of the above effect may be found in the fact that in milk the buffer index is much less when the milk is fresh, at pH about 6.6, than when the acidity has increased to approximately pH 5.2. Consequently, it may be expected that in the

first 3 hours in the press, small amounts of acid can produce relatively large changes in concentration of hydrogen ions.

The pH of the cheese after 21 hours serves as an indication of the activity of the lactobacilli in the cheese in the press. In large cheeses when the lactobacilli have been active, the pH at 21 hours will be about 5.0 to 5.1; if these bacteria were ineffective the pH at 21 hours may be 5.2 or over. Likewise the pH of the cheese after 3 hours is an indication of the activity of *S. thermophilus* ( $C_3$ ) in the cheese. A pH value of 6.05 to 6.1 at 3 hours would indicate that the activity of these streptococci was about normal. A pH of 5.6 at 3 hours would indicate great activity of  $C_3$  and a pH of 6.25 somewhat less than normal activity. These pH values will depend, of course, on the pH of the kettle milk and of the cheese curd at dipping time and should be considered as relative rather than absolute values.

Results at Washington, D. C., with small cheeses of about 50 pounds, green weight, indicate that the pH of the curd after three hours in the press should be 5.80 or less, and after twenty-one hours 5.15 to 5.30. These pH values would be obtained when *S. thermophilus* had been very active during the first hours that the cheese was in the press and when *L. helveticus* had been comparatively inactive during the first twenty-one hours. The cheeses were made from a mixed Jersey and Holstein milk with a pH of 6.5 to 6.6 and a titratable acidity of 0.17 to 0.18 per cent. Results were different with large cheeses of the size made in the cheese factories. The relationship between pH values of the large cheeses and quality of the cheeses after they were cured will be discussed more fully in a following paper (No. III of this series).

Figure 2 shows the pH curve and the growth curves of *S. thermophilus* and *Lactobacillus bulgaricus* Bergey, 1934 (Ga). The curves were made from data from a number of cheeses. The culture "Ga" is a lactobacillus grown with a mycoderm. This lactobacillus has a higher maximum temperature than 39a and starts to grow earlier in the cheese. It usually begins to increase in numbers about the 5th hour, but when a weak culture is used growth is considerably delayed. The growth of *S. thermophilus* in cheese made with the Ga culture is similar to its growth with 39a, but average results indicate that  $C_3$  reaches its maximum later in cheese containing Ga.

Table 2 shows the results from a cheese made with Ga and  $C_3$  cultures, with temperatures of the cheese where the samples were taken. It will be noted that Ga did not begin to grow until the temperature was below 44° C. 39a usually does not begin growth until the temperature falls below 43° C.

The temperature in the interior of large Swiss cheese will drop more slowly than in small ones. In a cheese of about 160 pounds, green weight,

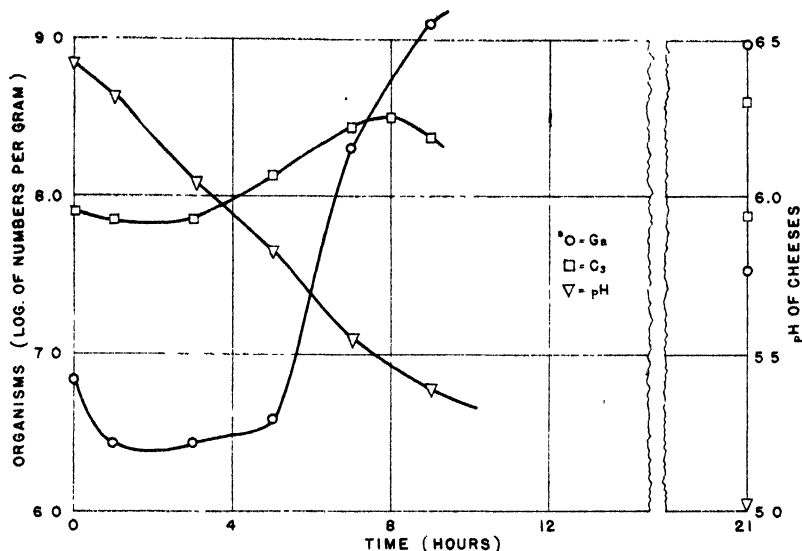


FIG. 2. GROWTH OF *L. bulgaricus* (Ga) AND *S. thermophilus* (Cs) IN SWISS CHEESE IN THE PRESS. AVERAGE RESULTS FROM A NUMBER OF CHEESES.

after a cooking temperature of 53.3° C. (128° F.), the temperatures six inches in from the hoop side were 50.5° C. at dipping time, 50° C. after 3 hours, 47° C. after 7 hours, and 39° C. after 21 hours. Both 39a and Ga begin to grow later in these large cheeses than in the smaller (Washington) cheeses. Results of counts on large cheeses at the Grove City Creamery showed that the Cs organisms were in about the same numbers during the early hours in the press as in the smaller Washington cheeses, but that the 39a organisms started to grow about two hours later in the larger cheeses. A common practice in Swiss cheesemaking is to add cool water or whey to the kettle contents just before the curd is dipped out. This reduces the temperature of the curd and makes it more like that of the curd of freshly dipped small cheeses.

#### GAS FORMING BACTERIA

As has been reported in a previous paper (5) organisms of the *Escherichia-Aerobacter* group are able to grow in the kettle contents during the Swiss cheese making process if they are present in large enough numbers in the original milk. That gas forming bacteria of this group are able to grow in the cheese in the press and cause "sounding" or even bloating is a well known fact. Dorner (2) reports that *Bact. aerogenes* is harmful to Swiss cheese and that *Bact. coli* is comparatively harmless. Similar results have been obtained at the Washington laboratories in

TABLE 4  
*Effect of added Escherichia communior on cheese with and without added S. thermophilus starter*

CHEESE NO.	CULTURES ADDED			EYES	TEXTURE	GLASS AND CHECKING	FLAVOR	SCORE	GRADE
	<i>L. helveticus</i>	<i>S. thermophilus</i>	<i>E. communior</i>						
877	Per cent 1/12	Per cent 1/12	Per cent 1/60	Too many; round clean Overset, irregular	Fair Fair	Trace None	Good Sharp	83 68.5	Good No. 1 No. 2
877-1	1/12		1/60						
878	1/12	1/12	1/120	Overset, round, regular	Fair	Checked end None	Good Sharp	78 68	Fair No. 1 No. 2
878-1	1/12		1/120	Nearly nissler	Fair				
879	1/12	1/12	1/300	Numerous, small, clean	Fair	None None	Good Sharp	79 68.5	Fair No. 1 No. 2
879-1	1/12		1/300	Overset, collapsed	Fair				
880	1/12	1/12	1/300	Round, clean, regular	Fair	Trace at end	Good	82	Good No. 1 No. 2
880-1	1/12		1/300	Small, irregular, gassy	Fair	None	Biting	68	

experiments in which a culture of *Escherichia communior* or of *Aerobacter aerogenes* was added to the kettle milk. Both *S. thermophilus* and *L. helveticus* starters were added to the control cheeses but the streptococcus culture was omitted from the test cheeses. Results of the experiments with *E. communior* are shown in Table 4 in which the description of four typical pairs of cheeses of the series at time of cutting is given. All of the cheeses which received gas organisms but no *S. thermophilus* starter were gassy or bloated in the press. The results showed that *E. communior* did not harm the cheeses when proper starter cultures were used. Figure 3

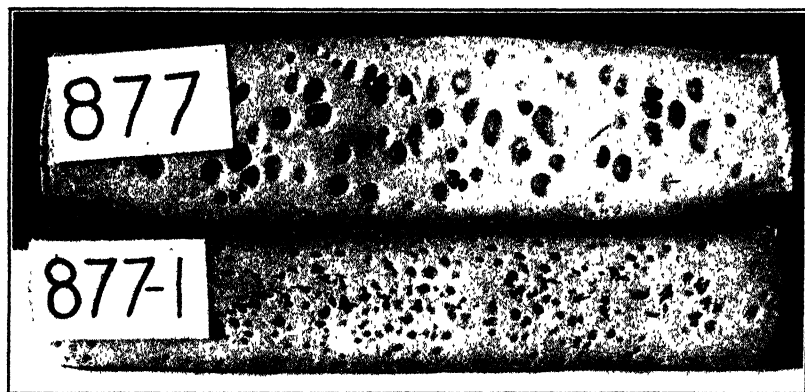


FIG. 3. PAIR OF THREE MONTHS OLD CHEESES MADE AT WASHINGTON, D. C. UPPER (CHEESE RECEIVED *S. thermophilus* CULTURE; LOWER RECEIVED NONE. *Escherichia communior* HAD BEEN ADDED TO THE MILK FROM WHICH THE CHEESES WERE MADE.

shows the No. 877 pair of cheeses when cut. In Table 5 are shown the results of similar experiments in which *Aerobacter aerogenes* was used instead of *E. communior*. All cheeses without added *S. thermophilus* were bloated in the press, while cheeses to which this starter had been added showed no signs of gas in the cheese in the press. Most of the cheeses, however, regardless of the starters added were of poor quality. This has been true of all cheeses to which very many *A. aerogenes* organisms have been added, regardless of the starters used and whether or not gas production has been suppressed while the cheese was in the press. In all experiments at these laboratories it has been possible, by the use of good starters, to suppress gas formation which would otherwise result from addition of *A. aerogenes* organisms, but the resulting cheeses have been consistently poor in grade. Figure 4 shows a pair of cheeses cut after 21 hours in the press. Cheese No. 744 received no *S. thermophilus* starter and was bloated in the press. Table 3 shows typical counts of gas and starter organisms in a cheese made from milk to which *A. aerogenes* had

TABLE 5  
*Effect of added Aerobacter aerogenes on cheeses with and without added S. thermophilus starter*

CHEESE NO.	CULTURES ADDED			EYES	TEXTURE	GLASS AND CHIPPING	FLAVOR	SCORE	GRADE
	L helveticus	S thermophilus	A aerogenes						
885	1/12	1/12	1/1000	Few, clean, round, fair	Fair	Both	Sour	73	Special
885-1	1/12		1/1000	Gassy edge	Fair	None	Sl. rancid	73	Special
886	1/12	1/12	1/900	Few eyes, fair	Good	Many checks	Fair	68	No. 2
886-1	1/12		1/900	Gassy edge	Good	None	Sl. rancid	75	Poor No. 1
887	1/12	1/12	1/60	Few eyes, gassy edge, Round, clean	Fair	Checks	Sour	68	No. 2
887-1	1/12		1/60		Fair	None	Sharp	77	Poor No. 1
888	1/12	1/12	1/60	Few eyes	Poor	None	Flat, sour	67.5	No. 2
888-1	1/12		1/60	Few eyes	Poor	None	Flat, sour	67.5	No. 2

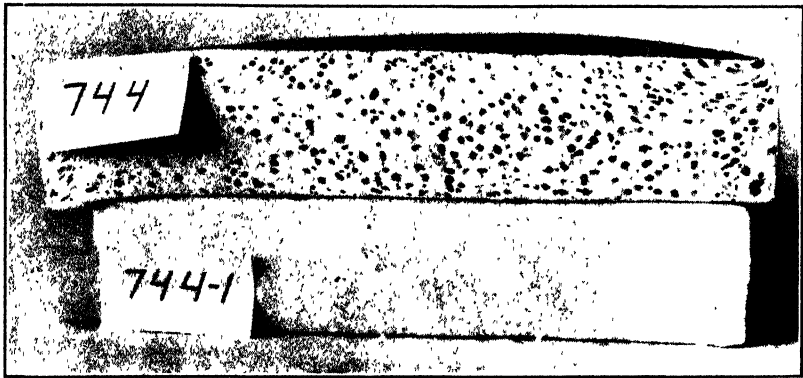


FIG. 4. PAIR OF ONE DAY OLD CHEESES MADE AT WASHINGTON, D. C. THE LOWER CHEESE RECEIVED *S. thermophilus* STARTER; THE UPPER CHEESE RECEIVED NONE. *Aerobacter aerogenes* HAD BEEN ADDED TO THE MILK FROM WHICH THE CHEESES WERE MADE.

been added. The gas bacteria were high in number just before the curd was dipped but decreased rapidly in the cheese and were not found after 3 hours in the press.

It has been our experience in cheese factories that the activity of the lactobacillus starter is also important in restraining the growth of gas-forming bacteria in cheese in the press. At the Grove City Creamery the cheeses usually "sound" or show evidences of gas formation when the cheese has a high pH value after 21 hours in the press, an indication of inactivity of the lactobacillus starter.

The anaerobic gas-forming bacteria of the *Clostridium* group do not usually grow enough in cheese in the press to cause evident changes at that time. Heavy contamination of the milk with spores of these bacteria in combination with weak or inactive starter bacteria may cause gas formation in the press. Usually the action of these anaerobic gas-formers in the cheese takes place later.

#### OTHER BACTERIA

Counts on the propionic acid bacteria, *Propionibacterium shermanii*, show that these organisms do not grow in the cheese in the press and in fact may decrease slightly in numbers. Other lactate-fermenting bacteria are able to grow in cheese in the press, however, and under some conditions may be present in large numbers 21 hours after dipping. It was reported in a previous paper (5) that some of these organisms can grow in the kettle contents. Some of them can grow still better in cheese in the press. Counts of tetracocci and of rod-shaped bacteria of the *Lactobacillus casei* Bergey, 1934, (*Bacillus casei* a v. Freudenreich) type in a

large number of cheeses showed that the tetracocci increased from 6,000–70,000, average 10,000 per cubic centimeter, in the kettle milk to 10,000–1,000,000, average 100,000 per gram, after 21 hours in the press. These tetracocci resemble those described by Orla-Jensen (8) and by him given the genus name of *Tetracoccus*. These organisms will be discussed in more detail in a following paper. When these bacteria are added to the kettle milk they sometimes reach large numbers in cheeses after 21 hours in the press. As many as a billion tetracocci or *L. casei* organisms per gram have been found in individual cheeses.

The growth of *S. lactis* is stopped by cooking temperatures in the kettle, as was shown in a previous paper (5). Counts of these organisms in the press show that even if they are present in large numbers when the curd is dipped they decrease rapidly in numbers during the early hours of the cheese in the press and are few in number at the end of 21 hours.

Organisms of the *Lactobacillus lactis* Bergey, 1934, and *Lactobacillus longus* Bergey, 1934, types were seldom found in the cheese in the press and then usually when unsterilized kettle whey had been used as a starter. As many as a billion of these organisms per gram have been found in 21-hour old cheese made with unsterilized whey starter. These cheeses were all of poor grade when cut.

#### DISCUSSION

It is evident that both *Streptococcus thermophilus* and a lactobacillus starter are important in cheese in the press. The streptococci are needed to produce an increase in acidity during the first hours in the press. Their action is almost complete after about eight hours and, unless the lactobacillus starts to grow at or before this time, opportunity is given for the growth of undesirable bacteria. An effective lactobacillus culture will continue to increase the acidity and bring the pH value of the cheese down toward 5.0 as a minimum.

Experiments reported in this paper have shown that a good *S. thermophilus* starter will suppress gassiness or bloating of the cheese in the press. Experience has shown that an effective lactobacillus starter is also necessary to keep down the gas-forming bacteria. A culture like Ga which starts to grow earlier than the 39a culture, and which causes a more rapid development of acidity in the cheese would be a more effective starter organism for combatting gas than 39a. Too rapid a production of acid in the cheese in the press, however, is harmful to the quality of the ripened cheese as will be shown in a following paper. Consequently, the addition of large amounts of starter may suppress gas formation in the cheese in the press but may cause other defects in the cheese. As has been stated above, the presence of large numbers of *A. aerogenes* bacteria in the kettle

milk usually foretells a cheese of poor quality, even when the gas formation in cheese in the press has been inhibited. Efforts must be made to keep the numbers of *A. aerogenes* in milk at a minimum if cheese of good quality is to be made.

The maximum acidity attained by the cheese in the first day is of importance in determining the fermentation which will take place subsequently in the cheese. If acid production has been too rapid during the first hours in the press, as indicated by a rapid drop in pH values of the interior of the cheese and too rapid drainage, then the pH at 21 hours will usually be high. The acidity of this cheese will never become as great as it should and, as a consequence, eyes will probably form too early and in too great numbers and the cheese will become overset. If, on the other hand, the early acid production is too slow, which tends to result in slow or insufficient drainage, the acidity of the interior of the cheese at 21 hours is likely to be high; in such a case eye formation will be delayed and the eyes may be too few, too small, or even absent ("blind" cheese). The regulation of the growth and action of the starter bacteria in the cheese in the press affords a means of controlling to a great extent the changes in the cheese both in the press and later in the curing rooms. It must be realized, of course, that proper use of starters will not correct defects due to poor milk, incorrect manufacturing methods, or improper care in the cellars.

#### SUMMARY

A study has been made of the bacteriology of Swiss cheese from the time the curd was dipped from the kettle until 21 hours later.

Small cheeses with a green weight of about 55 pounds were used in most of the experiments, but results have been compared to those with large cheeses.

A summary of results showed:

*S. thermophilus* usually started to grow within three or four hours after dipping and increased in numbers rapidly until the sixth or eighth hour. Thereafter the increase in numbers was slow and in some cases the numbers decreased gradually until the twenty-first hour.

*L. helveticus* (39a) usually decreased slowly in numbers until about the sixth to eighth hour in the press and then increased at a fairly rapid rate. The numbers after 21 hours were high with an active starter and low with a weak one.

*L. bulgaricus* (Ga) usually decreased in numbers until about the fifth hour after dipping when a rapid increase in numbers took place.

The pH of the cheese usually dropped more rapidly during the first seven or eight hours than during the later hours. This early drop in pH evidently was due chiefly to the action of the *S. thermophilus* organisms

present. Most of the cells of *S. thermophilus* had a diameter 3 or 4 times and a volume approximately 27 or 64 times as great as that of cells grown at ordinary temperatures. This is one explanation for the large fermentative activity per cell during the first hours of the cheese in the press. Moreover, small amounts of acid can produce relatively large changes in pH during the early hours in the press, since the buffer value is relatively small in the early pH range.

The pH of the cheese at three and at twenty-one hours after dipping may serve as an indication of the effectiveness of the *S. thermophilus* and lactobacillus starters, respectively. The relative change in pH is more significant than the actual pH value.

The action of *S. thermophilus* is about the same in large cheeses as in small, but the growth and action of the lactobacillus cultures usually begin an hour or two later in the larger cheeses, because the interior cools more slowly than in smaller cheeses.

Gas formation and growth by either *Escherichia communior* or *Aerobacter aerogenes* can be suppressed in cheese in the press by use of active starters. If *A. aerogenes* has been present in large numbers in the kettle contents, however, a poor quality cheese will probably result.

*Propionibacterium shermanii* did not grow in cheese in the press. Bacteria of the tetracoccus or *Lactobacillus casei* types usually increased in numbers, if present, and under some conditions attained large numbers by the twenty-first hour.

*C. lactis* died off rapidly in cheese in the press. Organisms of the *Lactobacillus lactis* and *Lactobacillus longus* types were able to grow rapidly but usually were not present in large numbers.

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# INFLUENCE OF SEASON AND ADVANCING LACTATION ON BUTTERFAT CONTENT OF JERSEY MILK

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## INTRODUCTION

Environment and stage of lactation are two factors affecting the fat content of cow's milk. The environmental conditions in Florida—seasonal ranges in temperature, distribution of rainfall and length of grazing season—differ from those of other latitudes. Because of these differences in environment, it was desirable to study the variations in the fat content of cow's milk in this latitude.

## THE STATION HERD AND ITS ENVIRONMENT

The dairy cattle owned by the Florida station are located at Gainesville, in the center of the north end of the Florida peninsula, some sixty miles from the Atlantic Ocean and from the Gulf of Mexico, at an altitude of about 176 feet above sea-level, between 29° and 30° North latitude. The temperatures from 1917 to 1932, inclusive, ranged from an average mean minimum of 46.5° F. in January, to a mean maximum of 90.7° in August. An average annual rainfall of 51.03 inches was reported at Gainesville during these years, of which 27.65 inches fell during June to September, inclusive. The dairy herd always has been limited in numbers of cows. The dairy barn has 36 stanchions, which seldom were all filled. During the earlier years, some high grade Jerseys were in the herd, but were replaced with registered Jerseys in the course of time.

Cows have grazed over the same pasture during this period of years. They were allowed access to pasture daily throughout the year, in the majority of seasons, and were stabled in an open barn only during the milking hours. Silage was fed to supplement pastures, almost to the exclusion of hay, prior to 1929. Since 1929, a very limited amount of alfalfa hay was fed to the higher producing cows. Feeding practices were reasonably uniform otherwise, except with regard to mineral supply, as was pointed out in Florida station technical bulletin 262.

## REVIEW OF LITERATURE

The seasonal influence on butterfat content of milk was observed by Eckles (2) to be independent of feeding practices. "The tendency is for the tests to reach the low point in early summer, usually June, and the highest point in December or January." He (4) stated later that "The

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effect of the season is apparently the result of weather conditions, especially heat and humidity. . . . during a period of hot, humid weather the percentage of fat is depressed, while if the conditions are . . . dry and cool, the test is increased." With cows fed mainly on alfalfa hay, Headley (8) observed the high average butterfat test in November and the low test in July. He stated that "These results show conclusively that the fat content of milk varies with the season, regardless of the kind of ration used." Baker and Cranfield (1) in a study conducted at milk depots in England, found the highest test in November, and the low average test in June. Hitteher (10) in a study of milk of 16 Holstein cows, noted that in all cases there was a rise in butterfat content of the milk in the month of September, probably due to environment.

Ragsdale and Brody (13) observed 10 cows over 41 days (March 13 to April 22) at the Missouri station. With mean daily temperature ranges between 27 and 70° F., there was a depression of almost 0.2 per cent in the average fat content of the milk, for an increase of 10° F. between the observed temperatures. Hays (7) noted a depression of 0.079 per cent fat per 10 degrees rise in mean temperature over 258 days, from January, 1924, when the entire Missouri station dairy herd was studied under uncontrolled conditions. With two Jersey cows kept in rooms with temperatures maintained more uniformly over periods of about one week duration, the average depression of 0.095 per cent in fat test per 10 degrees rise in temperature, was noted in seven trials between 27, and 92.7° F. In the temperature range between 27 and 72.5 degrees, an average depression of 0.189 per cent fat occurred for each 10 degrees depression in temperature. Above 70° F., an unexpected rise in fat test occurred, perhaps attributable to an increased rate of metabolism with the higher temperature. Temperature, Hays concluded, is a major factor in the seasonal variation in percentage of fat in cow's milk.

Weaver and Matthews (17) analyzed one year's records of dairy cows at the Iowa station, and calculated by application of regression equations that for each degree of increase in atmospheric temperature outside the dairy barn the fat percentage in Ayrshire milk was decreased 0.0017 per cent; in Guernsey milk, 0.0103 per cent; in Holstein milk, 0.0063 per cent; and in Jersey milk, 0.0066 per cent.

It is generally recognized that milk tends to become richer in fat as lactation advances. However, this increase is not continuous from date of calving. Breeds differ as to duration of an interval following parturition, during which a high butterfat test occurs temporarily, this period usually being one-half to three months in length. Eckles and Shaw (4) state that "The per cent of fat on the average, declined during the first three months, followed by a period of 4 or 5 months with but little change. From this point a rapid increase was found to the end of the lactation period."

Hills (9) studied 30 private herds in Vermont, and additional lactation records from herds in Minnesota and New York. He reported that, in general, changes in the fat tests were inverse to changes in temperatures. Also, that the lowest fat tests with two-thirds of the cows, occurred within 2 to 4 months after calving, and the highest tests after the seventh month of lactation. Hogstrom (11) studied 822 lactations, principally by Ayrshire cows, and found that after omitting the colostrum period, the percentage of fat was higher the first month, then decreased until the third month, when it reached the minimum. From there, the butterfat tests increased gradually, attaining the maximum at the close of lactation. Speir (16) reported that the highest fat content of the milk from a group of almost 400 Ayrshire cows was during the first week after calving, and that the lowest average test was during the fourth week, followed by a gradual increase to the end of the lactation. Linfield (12) tabulated the lactations of 16 cows of several breeds in the Utah station herd, and concluded that the percentage of fat decreased slightly in the second month, then gradually increased until the ninth month, after which the rate of increase was more rapid, until the end of the lactation.

Eckles (3) pointed out from a study of 3,154 lactations representing several breeds of dairy cows, that there was a slight decline from the first to second month after calving, followed by a gradual increase to the twelfth month. Moreover, he stated that ". . . the seasonal influence modified the variation in fat content of milk materially, and in fact is a greater force than stage of lactation, except for the rapid increase as the cow is going dry."

#### EXPERIMENTAL METHODS AND RESULTS

From the Florida station herd, 293 lactations were obtained by registered Jersey cows. The cows were milked twice daily, and butterfat tests had been made at seven or more monthly intervals during each lactation. The preceding calf had been dropped normally, and no illness or other condition was known which might have an abnormal effect upon lactation.

The 293 lactations were assembled into groups according to the month in which the cows freshened. These groups ranged from 15 cows that calved during May, to 40 during October. The average fat tests were determined by months for each group. All fat determinations had been made by the Babcock method. The number of monthly butterfat tests ranged between 219 in September and October, and 266 during March. These group averages were assembled in such a manner as to show the calendar month of each test, as well as the number of months after calving, giving the group averages equal weighting.

The method of arranging average butterfat tests in 144 cells (twelve rows of cells for calendar months and twelve rows for the successive months

centage in the milk of Jersey cows than do the environmental (seasonal) factors.

#### STATISTICAL SIGNIFICANCE

Fisher's "Z" test (6) as modified by Snedecor (15), was used to test for significant contributions to variance in butterfat percentages in Jersey milk by season of the year and advancing lactation. The ratio of the between-month-after-calving mean square to the remainder mean square is 25.76:1. The ratio of the between-calendar-months mean square to the remainder mean square is 15.24:1. These two "F" values are much greater than the "F" values for the point of one per cent probability, as given in Snedecor's tables of "F" values. Both are highly significant. The ranges in fat percentage between months after calving are seen to be more highly significant, and of greater magnitude, than those for different calendar months. These relationships are shown in table 2.

TABLE 2  
*Contribution of month-after-calving and of calendar-month to total variance in percentages of butterfat in the milk of Jersey cows*

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	"F" VALUE
Total	144 - 1 = 143	30.832800		
Between months after calving	12 - 1 = 11	15.272347	1.38839518	25.762
Between calendar months	12 - 1 = 11	9.039401	.82176373	15.248
Remainder	11 × 11 = 121	6.521052	.05389299	

#### TEMPERATURE AFFECTS FAT PERCENTAGE

Meteorological data from the observatory of the United States Weather Bureau were assembled for the same period of years over which the butterfat records were obtained. The mean maximum and mean minimum temperatures were averaged, as shown in table 3, and used in analysing the relative influence of temperature upon butterfat percentage in the milk of Jersey cows. The method of analysing the butterfat percentages, as previously described, permitted the data used here to be practically independent of modifying influences due to advancing stage of lactation.

Between the range of 57 and 81° F., average mean temperatures for the different calendar months, there was a spread of approximately 0.70 per cent butterfat in the milk of these Jersey cows. Ezekial's formulae 8, 9 and 10 (5) were applied to these data, on the assumption that within this range of temperatures, the variation in fat percentage was a straight line function. For each increase of 10° F. between 57° and 81°, a decrease of 0.31 per cent fat was found. The results of this analysis are shown in figure 2, which is based upon data given in table 3.

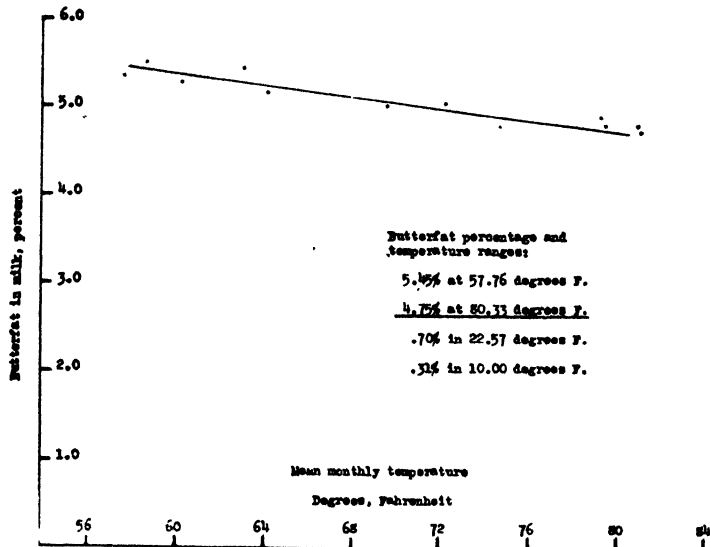


FIG. 2. ASSUMING THAT THE CHANGE OF BUTTERFAT PERCENTAGE WITH TEMPERATURE IS A STRAIGHT LINE FUNCTION WITHIN CERTAIN LIMITS, IT WAS CALCULATED FROM JERSEY BUTTERFAT TESTS ACCUMULATED OVER 16 YEARS, THAT A DECREASE OF 0.031 PER CENT FAT OCCURRED PER DEGREE INCREASE IN TEMPERATURE BETWEEN 57 AND 81°.

It may be mentioned in this connection that Hays (7) suggested from a study of fewer records of animals under more closely observed temperatures, but with the influence of advancing stage of lactation only partially

TABLE 3

*The relationship between mean temperatures, average rainfall and monthly butterfat percentages in Jersey milk at Gainesville, Florida, 1917 to 1932*

MONTH	MEAN TEMPERATURE			AVERAGE RAINFALL INCHES	AVERAGE BUTTERFAT PERCENTAGE
	Maximum degrees	Minimum degrees	Average degrees		
January	68.7	46.5	57.6	3.15	5.33
February	71.7	48.7	60.2	2.83	5.27
March	75.4	52.8	64.1	3.62	5.17
April	81.4	57.4	69.4	3.23	5.03
May	86.5	62.5	74.5	3.46	4.80
June	89.7	68.8	79.25	6.96	4.83
July	90.6	70.8	80.7	9.07	4.81
August	90.7	70.8	80.75	6.26	4.76
September	89.0	69.1	79.05	5.36	4.92
October	82.9	61.2	72.05	2.56	5.06
November	74.6	51.4	63.0	1.94	5.44
December	69.5	47.6	58.55	2.59	5.49

eliminated, that a curvilinear function may appear at some point between 70 and 90° F.

#### SEASON AND STAGE OF LACTATION, AS RELATED TO BREED AND LOCALITY

A search of the literature yielded six sets of butterfat tests from the Missouri (2, 14), Iowa (2) and Tennessee (18) stations and Sweden (11) in such form that the same method of analysis could be applied for variations due to season and stage of lactation. These data were for the Ayrshire, Guernsey, Holstein and Jersey breeds and for one large group of mixed breeds. A comparison is shown in table 4, of the results obtained by applying this method of analysis of variance to the data from these sources.

It may be noted that, in general, the low average butterfat test occurred during the second month after calving, with Holsteins, Guernseys and Jerseys, and in the third month with Ayrshires.

The low butterfat tests in the northern hemisphere occurred during the summer—June, July, or August. High tests were during the winter months of November, December, or January. The general trends of butterfat tests through the seasons and with advancing lactation under the Florida environment, are shown in figure 1.

Thus the general trends in fat percentage in the milk of Jersey cows in Florida are similar to those noted with the other breeds in more northerly latitudes. The magnitude of the variations in other latitudes differed with the several breeds, being greater as a rule with the breeds having a typically higher percentage of butterfat in the milk. In each instance where the records of a single breed were involved the range in variation of fat percentage, due to advance in stage of lactation, was significantly greater than that due to season of the year, as seen in the corresponding "F" values in table 4. Stating these results in other words, environment exerted less influence upon the fat percentage in cow's milk than did the internal or physiological factors due to advance in stage of lactation.

The only exception to the above generalization, seen in the groups of data analysed in table 4, is that of 240 records representing mixed breeds at the Missouri and Iowa stations. It is believed that the few numbers representing certain breeds, and the uneven distribution of the breeds within each cell, were sufficient to render the relative influences inconclusive because of the unequal weighting of breeds. Even in this instance, the fat percentages tended to follow the general trends of variation according to season and stage of lactation, as noted in the separate breed groups.

#### DISCUSSION OF RESULTS

The butterfat content of Jersey milk ranged, with advancing lactation, between 4.594 per cent in the second month after calving, and 5.555 per cent

TABLE 4  
*Comparison of stage of lactation and of season of the year, as these influence butterfat percentage in the milk of dairy cows in several regions*

SOURCE	BREED	NUM- BER	AVERAGE BUTTERFAT PERCENTAGE IN MONTHS AFTER CALVING												"P" VALUES
			1	2	3	4	5	6	7	8	9	10	11	12	
Florida Missouri	Jersey	293	4.61	4.59	4.68	4.83	5.02	4.99	5.15	5.29	5.29	5.38	5.50	5.56	25.76
	Jersey	299	4.83	4.93	5.13	5.19	5.30	5.45	5.45	5.52	5.61	5.62	5.75	5.70	25.95
	Holstein	95	3.24	2.93	3.00	3.06	3.01	3.08	3.11	3.21	3.19	3.27	3.33	3.44	7.73
	Guernsey	3,763	4.63	4.59	4.71	4.84	4.97	5.08	5.16	5.21	5.29	5.39	5.49	5.59	488.75
United States Missouri and Iowa Sweden	Mixed	240	4.29	4.28	4.34	4.35	4.45	4.51	4.60	4.62	4.73	4.77	4.83	4.83	7.94
	Ayrshire	891	3.79	3.57	3.50	3.54	3.64	3.68	3.78	3.90	3.96	4.13	4.20	4.17	29.51
AVERAGE BUTTERFAT PERCENTAGE IN CALENDAR MONTHS															
Florida Missouri	Jersey	293	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
	Jersey	299	5.33	5.27	5.17	5.03	4.80	4.83	4.81	4.76	4.92	5.06	5.44	5.49	15.25
	Holstein	95	5.67	5.50	5.34	5.31	5.21	5.17	5.03	5.03	5.37	5.55	5.58	5.67	15.34
	Guernsey	3,763	3.19	3.26	3.26	3.20	3.13	3.00	3.03	3.05	3.05	3.12	3.30	3.25	3.70
United States Missouri and Iowa Sweden	Guernsey	3,763	5.22	5.17	5.13	5.07	5.01	4.95	4.89	4.88	5.02	5.15	5.22	5.24	70.48
	Mixed	240	4.76	4.68	4.66	4.58	4.43	4.31	4.24	4.23	4.34	4.62	4.84	4.90	9.96
	Ayrshire	891	3.86	3.83	3.87	3.73	3.71	3.59	3.62	3.81	3.85	4.00	4.05	3.93	9.01

in the twelfth month after calving. The range for seasonal influence was between 4.760 per cent in August, and 5.464 per cent in December. By statistical analyses, these influences were measured separately in another way, by which the "F" values for variance were found to be 25.76 for between-months-after-calving, in contrast to 15.24 for between-calendar-months. ("Variance" is a term used by Fisher to denote the square of the standard deviation.) The arithmetical and analytical measures both indicate that the magnitude of physiological or internal influences affecting fat percentage in Jersey milk are appreciably greater than those exerted by the average external influences of environment existing in Florida. This observation is in accord with our analyses of the data obtained from separate breeds studied in more northerly latitudes.

#### SUMMARY AND CONCLUSIONS

The seasonal trend in fat percentage in the milk of Jersey cows in the more uniform environment of Florida was found to vary almost inversely with temperature. The lowest average butterfat tests occurred as a rule in August and the highest in December.

The average test of Jersey milk in the first month after calving was 4.605 per cent, dropped to 4.594 in the second month, and then increased steadily to the twelfth month.

Advancing lactation exerted a greater influence than did season of the year upon the percentage of fat in the milk of Jersey cows.

An average increase of 10° F. between monthly mean temperatures of 57° and 81° resulted in an average decrease of 0.31 per cent butterfat in Jersey milk, in a study of records obtained over a 16-year period.

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# BEHAVIOR OF CASEINATE SOLS IN A STUDY OF A HYSTERESIS-LIKE PHENOMENON IN THE RENNET COAGULATION OF HEATED MILK\*

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It is commonly known that heated milk does not react normally with rennet. That heat treatment is not always detrimental was first observed by Stassano and Talarico (1), who noticed that milk heated to 65° C. coagulates more rapidly than raw milk. Similar observations were made by Rupp (2), and Mattick and Hallett (3), the latter investigators also observing an apparent hysteresis-like phenomenon.<sup>1</sup> They found that when milk is heated for 30 minutes at 105° F. to 141° F. and rapidly cooled to 84° F. the first effect is increased coagulability, but the milk gradually loses coagulability as the interval between heating and addition of rennet lengthens. When the milk is heated above 145° F. the loss of coagulability is immediate and continues to increase as rennet addition is delayed, reaching a maximum after 5 hours. Moir (4) made a similar observation with flash pasteurized milk.

As Hammarsten (5) early demonstrated that only the calcium caseinate and calcium phosphate systems in milk are involved in the clotting by rennet, it appeared feasible to employ these two components as an artificial "milk" in the study of the hysteresis-like phenomenon of heated milks. It was hoped that some evidence could be secured as to the analogous fraction in natural milk responsible for the different behavior of heated milks toward rennet.

## COAGULABILITY OF HEAT-TREATED CALCIUM CASEINATE-COLLOIDAL CALCIUM PHOSPHATE "MILKS"

All of the "milks" were prepared to contain 2.5 per cent casein in order to approximate the quantity of casein in natural milk. The casein was prepared by the method of Van Slyke and Baker (6) as refined by Van Slyke (7). The concentration of calcium associated with the casein can be varied through a wide range as Porcher (8) has shown. By assuming that casein is combined with 1.15 per cent Ca at the pH of milk [Palmer and Richardson (9)] and that casein contains 0.85 per cent P [Hammarsten

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<sup>1</sup> Hysteresis is a term applied to colloidal systems that react according to the past treatment which they have received.

(10), Lehmann (11), Van Slyke (12)] the data on Van Slyke and Bosworth (13) may be recalculated to show that on the average 30 per cent of the total P and 43 per cent of the total Ca in natural milk occurs as colloidal mineral compounds. However, in the preparation of the artificial "milks" only about 77 per cent of these quantities were used, otherwise the system would coagulate spontaneously when warmed to 40° C.

The calcium caseinate-colloidal calcium phosphate complexes were prepared by triturating the casein in a small quantity of clear limewater; subsequent small additions of limewater were made until a thin paste was formed, whereupon a sufficient amount was then added to prepare an alkaline caseinate of pH about 10.5. After allowing this solution to stand for about an hour to insure complete peptization of the casein the pH was reduced to that of natural milk by the slow addition of 0.6 per cent  $\text{H}_3\text{PO}_4$  solution from a capillary-tipped pipette extending beneath the surface while the liquid was rapidly agitated by a motor driven stirrer. After dilution to the required volume with distilled water the "milk" was aged for 14 hours at 5° C. The following data illustrate a typical "milk": 25 gm. casein, 860 cc. clear limewater, 85 cc.  $\text{H}_3\text{PO}_4$  solution, 65 cc.  $\text{H}_2\text{O}$ ; pH 6.65. Eight different lots of "milk" were prepared by this method.

Later another method of preparation was adopted in which the pH did not exceed the limits of normal milk at any time. These systems produced coagulation curves similar to those prepared as above. A 5 per cent calcium caseinate sol was first prepared by triturating the casein in the usual way and adding sufficient limewater to produce a pH comparable to natural milk. After diluting the sol to about a 5 per cent casein content it was aged 14 hours at 5° C. Next a predetermined amount of limewater and 0.6 per cent  $\text{H}_3\text{PO}_4$  solution were slowly added simultaneously to the mechanically stirred caseinate sol from capillary-tipped pipettes. The acid solution was first diluted with sufficient distilled water to increase the complete complex to the desired volume. An example is 25 gm. casein, 345 cc. clear limewater, 150 cc. water; pH 6.72. To this aged sol were then added as described 300 cc. clear limewater, 65 cc. of 0.6 per cent  $\text{H}_3\text{PO}_4$  solution diluted with 140 cc. distilled water; pH 6.70. The "milk" was aged 14 hours at 5° C. before using.

Since only heat-treated portions not coagulated by the heat treatment itself were desired a sample of each "milk" was heated at 85° C. or higher for 10 minutes, cooled and observed in an ultramicroscope. Any clustering observed in the colloidal suspension could usually be prevented by a short dialysis of the unheated "milk." The heating of the artificial "milks" was carried out as follows: Two portions of each "milk" were placed in loosely stoppered flasks; one flask was rapidly heated in a water bath to 60° C., the other to 85° C. and maintained at the respective temperatures for 30 minutes. Both portions were then promptly cooled to 35° C. and

samples removed for immediate coagulation; the remainder was cooled to approximately 20° C. Samples of the latter were tested for rennet coagulability at 40, 70 and 130 minute intervals after the end of the heating period. All coagulation tests were made at 40° C.

As a result of the heat-treatment at 60° C. and 85° C. the pH of the "milks" decreased slightly, the greatest lowering always occurring in the

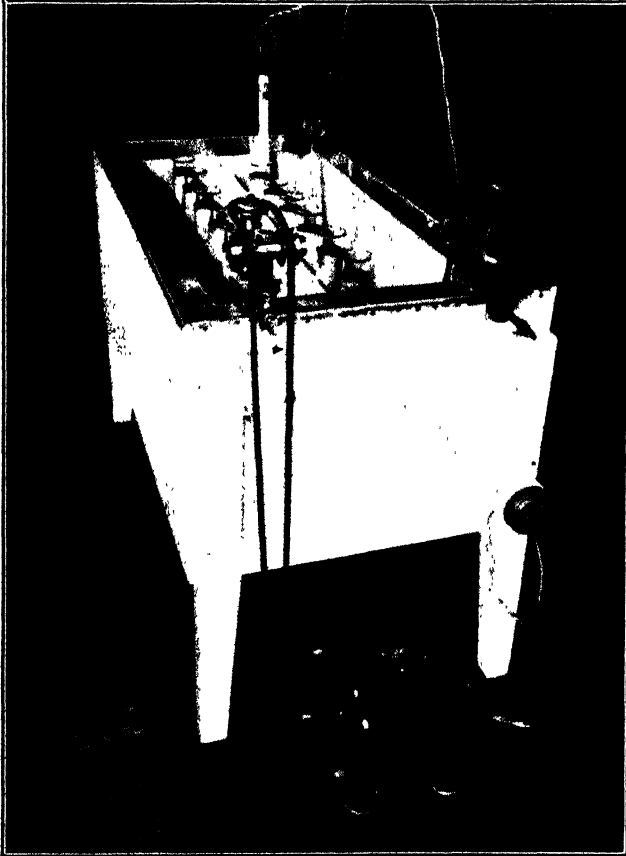


FIG. 1. Electrically heated and controlled water bath in which all samples were coagulated.

portion heated at 85° C. However, in no case was the decrease greater than pH 0.05 and usually only pH 0.03 as the typical example in Table 1, Exp. A indicates. In occasional experiments pH measurements were made on the control and the heated portions at the beginning and again at the completion of the experiment. Data are given on this point in the following

typical example, the first values given being the initial pH and the second the pH two and one-half hours later, at the completion of the experiment: unheated calcium caseinate-phosphate "milk" pH 6.70 and 6.70; after heating to 60° C. pH 6.66 and 6.662; after heating to 85° C. pH 6.65 and 6.65.

Fig. 1 shows the electrically heated water bath in which all samples were coagulated. In this figure are visible the thermo-regulator, heating element, stirrer and the series of copper tubes containing the 100 cc. glass coagulation tubes. The latter are fitted with glass-tipped rubber tubing that will extend through an opening in the bottom of the bath; also a rubber stopper that will close the opening in the bottom of the bath thus permitting a water contact between the glass and copper tubes. Each tube is closed with a screw clamp close to the bottom of the bath.

Fifty cc. samples of "milk" were pipetted into the coagulation tubes 5 minutes before the rennet was added; a predetermined amount of  $\text{CaCl}_2$  solution was put in next and the mixture stirred occasionally. As soon as the "milk" had reached the temperature of the bath rennet was added, a stop-watch started and the mixture stirred for 30 seconds. The quantities of  $\text{CaCl}_2$  solution and rennet were adjusted to cause coagulation of the unheated controls in 3 to 5 minutes; these same quantities were added to the heated portions. The screw clamp was opened slightly at the end of 3 minutes and a fine stream of "milk" allowed to run into a glass beaker. As soon as flakes of curd were visible on the side of the beaker the watch was stopped and the coagulation time recorded. All experiments were carried out in duplicate.

With some of the unheated "milks" the concentrations of  $\text{CaCl}_2$  solution and rennet employed produced merely a coagulation within the desired time whereas with others the "milk" clotted although no attempt was made to insure clots. The diluted rennet solution was held in very cold water so as to reduce inactivation to a minimum. A typical coagulation curve of a heat-treated calcium caseinate-phosphate complex is shown in Fig. 2A. The curve indicates that this system exhibits a hysteresis-like phenomenon when heated to 60° C. and 85° C. The pH of the original "milk" was 6.82; after heating to 60° C. pH 6.80 and after heating to 85° C. pH 6.785.

*Effect of Heat-treatment on Casein.*—According to Marni (14), the effects of heating caseinate solutions are not deep-seated, for he prepared an artificial "milk" which coagulated normally with rennet by using casein isolated from a caseinate solution which had been boiled. We performed a similar experiment. A calcium caseinate-phosphate "milk" was first prepared using 100 gm. casein, 3600 cc. clear limewater, 300 cc. 0.6 per cent.  $\text{H}_3\text{PO}_4$  solution; final volume 4000 cc., pH 6.61. This "milk" required

<sup>2</sup> Hansen's Liquid Cheese Rennet was used as a stock solution.

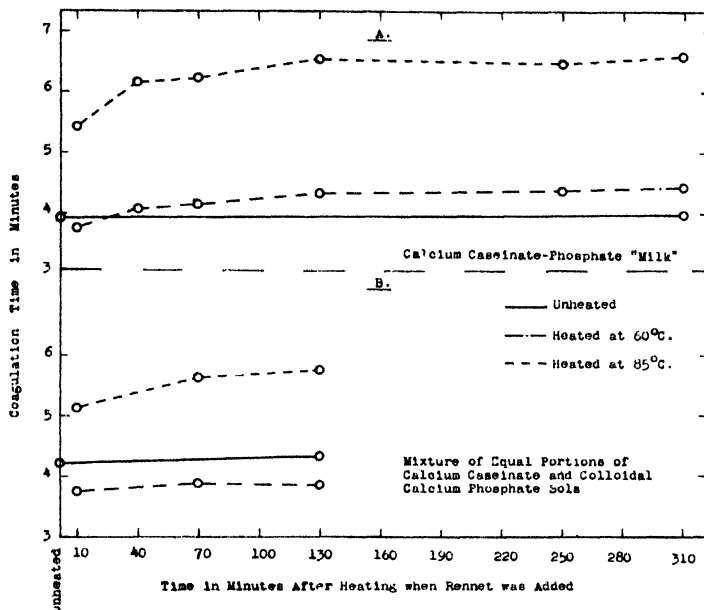


FIG. 2. A. Typical coagulation curves of a calcium caseinate-colloidal calcium phosphate "milk" coagulated at intervals after heating at 60° C. and 85° C. for 30 min. B. Typical coagulation curves of mixture of calcium caseinate and colloidal calcium phosphate sols coagulated at intervals after heating at 60° C. and 85° C. for 30 min.

dialysis before it would withstand boiling without showing clustering in the ultramicroscope; the pH after dialysis was 6.80. The product was next held in a boiling water bath for 30 minutes, cooled and aged 14 hours at 5° C. The casein was then isolated by the method of Van Slyke and Baker (6) and Van Slyke (7). Using this casein a new calcium caseinate-phosphate "milk" was prepared with 26.5 gm. casein, 950 cc. limewater and 76 cc. 0.6 per cent  $H_3PO_4$  solution; final volume 1060 cc., pH 6.72. A boiled sample was free from ultramicroscopic clustered particles. The coagulation times of the control and portions heated to 60° C. and 85° C. are given in Table 1, Exp. A, and show the same general trend as the "milks" shown in Fig. 2.

#### COAGULABILITY OF MIXTURES OF CALCIUM CASEINATE AND COLLOIDAL CALCIUM PHOSPHATE SOLS

A representative calcium caseinate sol was prepared by triturating 25 gm. casein in 422 cc. clear limewater. A dense milky sol resulted which was diluted to 500 cc. with distilled water; final pH 6.75. A colloidal calcium phosphate sol was prepared by dissolving 5 gm. of high grade gelatin in

TABLE 1  
Effects of heating various artificial "milks" on their coagulation with rennet

EXP. NO.	TYPE OF SYSTEM	FRACTION HEATED	HEATING TEMP.	PH OF HEATED FRACTION	COAGULATION TIME AT VARIOUS PERIODS AFTER HEATING					
					0	10 <sup>1</sup>	40	70	130	190
A.	Calcium Caseinate-Phosphate	Entire "Milk"	Unheated	6.72	3:20				3:19	
			60	6.70	3:23					
						3:14	3:20	3:35	3:41	
						3:15	3:28	3:38	3:44	
			85	6.69		5:00	5:34	5:50	6:20	
B.	Calcium Caseinate Calcium Phosphate Mixture	Calcium Caseinate	Unheated	6.72	4:00				3:54	
			60	6.71	4:01				3:58	
						4:06		3:58	3:50	
						4:00		3:55	3:47	
			85	6.70		4:03		3:57	3:53	
C.	Calcium Caseinate Calcium Phosphate Mixture	Calcium Phosphate	Unheated	6.60	4:49					4:55
			60	(6.69) <sup>2</sup> 5.94	4:50					
						5:00			4:56	5:00
				(6.59) <sup>2</sup> 5.71		5:00			5:02	5:04
			85	(6.55) <sup>2</sup> 5.71		4:29			4:26	4:23
D.	Calcium Caseinate	Entire Sol	Unheated	6.70	4:02				4:04	
			60	6.70	4:05				4:06	
						4:05	4:05	4:10	4:12	
						4:07	4:05	4:06	4:07	
			85	6.70		4:15	4:18	4:15	4:17	
E.	Calcium Caseinate-Arsenate	Entire "Milk"	Unheated	6.75	4:20				4:22	
			60	6.72	4:21				4:21	
						4:02	4:11	4:14	4:10	
						3:59	4:12	4:15	4:10	
			85	6.73		4:00	3:49	3:45	3:45	
F.	Calcium Caseinate-Oxalate	Entire "Milk"	Unheated	6.84	3:45				3:45	
			60	6.86	3:45				3:45	
						3:45	3:45	3:45	3:45	
						3:45	3:45	3:45	3:45	
			85	6.86		3:45	3:45	3:45	3:45	

<sup>1</sup> Approximately 10 minutes were required to cool the heated "milks" and adjust the first samples to the temperature of the water bath.

<sup>2</sup> pH of a mixture containing equal portions of unheated caseinate and heated phosphate sols.

430 cc. clear limewater, cooling the solution under tap water and partially neutralizing 56.5 cc. 0.6 per cent  $H_3PO_4$  solution with some of the gelatin-limewater solution until a slight precipitate began to form. This limewater-acid mixture was slowly added to the remainder of the mechanically stirred gelatin-limewater solution from a capillary-tipped pipette held beneath the surface. A stable colloidal suspension resulted which was diluted to 500 cc. with distilled water; pH 6.60.

*Heat-treating the Mixture.*—A mixture of equal portions of the calcium caseinate and calcium phosphate sols appeared like a calcium caseinate-calcium phosphate complex; the pH was 6.71. The coagulation curves of this mixture before and after heating at 60° C. and 85° C. are shown in Fig 2B and are similar to those of the complex. The pH of the portion after heating to 60° C. was 6.68; after heating to 85° C. pH 6.658.

*Heat-treating the Calcium Caseinate Fraction.*—In order to determine whether the hysteresis effects of heating involve the protein fraction or the inorganic phosphates or whether the presence of both is required a series of calcium caseinate sols were prepared as previously described and also a parallel series of colloidal calcium phosphate sols. The phosphate sols were made by adding calcium sucrate and  $H_3PO_4$  solutions simultaneously to a dilute gelatin solution. A typical sol consisted of 420 cc. 1.2 per cent gelatin solution, 10 cc. of a calcium sucrate solution containing 8.0 per cent  $Ca(OH)_2$  and 70 cc. 0.6 per cent  $H_3PO_4$  solution. Both the sucrate and acid solutions were diluted to 150 cc. with some of the gelatin solution and were then slowly added to the remainder of the mechanically stirred gelatin from capillary-tipped pipettes held beneath the surface of the liquid. The stable translucent sol had a pH 6.72.

In order to coagulate 50 cc. of a mixture of equal portions of caseinate and phosphate sols 1.0 cc. of 0.15N  $CaCl_2$  solution was required in addition to the rennet. In one series of experiments one-half of the necessary  $CaCl_2$  was added to the calcium caseinate sol before heating, but in the other series the  $CaCl_2$  was added to the mixed sols immediately before the coagulation test. Table 1, Exp. B shows the effects on coagulability with rennet when the calcium caseinate fraction was heated to 60° C. and 85° C. for 30 minutes before cooling and adding the colloidal calcium phosphate sol. The results show no significant effect on the coagulation time of the mixed sols.

*Heat-treating the Calcium Phosphate Fraction.*—Calcium caseinate and colloidal calcium phosphate sols were prepared in a manner similar to those just described. In this study the phosphate sols were previously heated and cooled before mixing with the calcium caseinate fraction for coagulation. Nine separate experiments were conducted in which four different lots of casein and calcium sucrate solutions were used. A typical experiment follows: A 5 per cent calcium caseinate sol made with 30 gm. casein and 415 cc. clear limewater had a pH of 6.80 after diluting to 600 cc. The phosphate sol was made with 485 cc. 1.24 per cent gelatin, 19.75 cc. calcium sucrate containing 5.3 per cent  $Ca(OH)_2$  and 94 cc. 0.6 per cent  $H_3PO_4$  solutions. Both the acid and base solutions were diluted to 150 cc. with some of the gelatin solution and then were added simultaneously to the remainder of the gelatin; pH 6.60.

All cases in which the colloidal calcium phosphate sols were heated a peptization<sup>3</sup> of colloidal material resulted, being especially marked at 85° C. The hydrogen-ion concentration was always increased by heat-treating these sols, the extent of the increase being directly proportional to the temperature. For example, in the sol just described after heating at 40° C. the pH was 6.37; at 60° C., 5.94 and at 85° C. 5.71. Similar but much less extensive effects were obtained after mixing the raw caseinate sol and the heated phosphate sols as shown in Table 1, Exp. C, (pH data in parentheses). This experiment shows the effects on coagulation when portions of the phosphate sol were heated at 60° C. and 85° C. for 30 minutes before cooling and adding the caseinate for rennet coagulation. The hysteresis-like effects of delayed rennet addition are absent. Although this experiment is representative of most of those performed, in two trials in which different lots of casein and calcium succrate were used to prepare the respective sols a decreased coagulability was observed which was so marked that it was apparent even when the phosphate fraction was heated at 40° C. and no coagulation curves were obtainable. The explanation has not yet been found for these two results.

#### COAGULABILITY OF HEAT-TREATED CALCIUM CASEINATE SOLS

Several investigators, including one of us, (15) have postulated that the effects of heating milk on its coagulation with rennet are to be explained on a basis of a disturbance in the colloidal calcium caseinate system. In order to determine whether artificial systems of calcium caseinate would exhibit the hysteresis-like behavior portions of several calcium caseinate sols were heated to 60° C. and 85° C. for 30 minutes, cooled and coagulated at 40° C. In some sols a portion of the  $\text{CaCl}_2$  required for coagulation was added before heating while in others it was added afterwards; this modification did not affect the coagulation times.

The caseinate systems produced a peculiar type of clot with rennet, the curd having a gummy or rubbery consistency which synerized rapidly. Also, considerably greater concentrations of  $\text{CaCl}_2$  were necessary than for the caseinate-phosphate complexes. One sol clotted with one cc. 0.35N  $\text{CaCl}_2$  solution but others required one cc. 0.48N  $\text{CaCl}_2$ . Table 1, Exp. D shows the typical behavior of the heated sols of calcium caseinate. Previous heating of these sols was practically without effect on their coagulability, there being no evidence of the hysteresis shown by the caseinate-phosphate complexes.

<sup>3</sup> Although the decreased colloidal stability was found to be closely associated with the increase in acidity, the previous colloidal stability could not be restored by addition of alkali to the previous pH. Example: Original pH of phosphate 6.81; after heating 85° C. 5.78; after addition of alkali 6.76. The presence of soluble electrolytes was found to be without influence inasmuch as dialysis failed to prevent both the decrease in colloidal stability and the drop in pH on heating.

COAGULABILITY OF CALCIUM CASEINATE COMPLEXES MADE WITH  
ARSENIC AND OXALIC ACIDS

Although Hammarsten (16) first stated that other acids should replace phosphoric in the preparation of artificial "milks" this hypothesis was developed by Porcher (8) and Marui (14). The former postulated that arsenic acid could be used on account of its periodic relationship to phosphoric. Accordingly, a few calcium caseinate-calcium arsenate "milks" were prepared and studies made of the coagulation of heated portions. These "milks" were prepared in general by triturating casein in limewater to pH about 10.50 and increasing the hydrogen-ion concentration by the slow addition of M 10  $\text{H AsO}_4$  solution. A representative "milk" contained 30 gm. casein, 1020 cc. clear limewater and 110 cc. M/10  $\text{H AsO}_4$  solution; after dilution to 1200 cc. the final pH was 6.68. A preliminary dialysis was necessary to prevent coagulation at 85° C.; the pH after dialysis had increased to 6.75. Although the "milk" was now stable at 85° C. it coagulated in boiling water.

These "milks" had the same appearance as the caseinate-phosphate complexes but required more  $\text{CaCl}_2$  solution as 50 cc. of "milk" required one cc. N/2  $\text{CaCl}_2$  solution for rennet coagulation at 40° C., thus resembling the calcium caseinate sols. Portions of the "milk" were heated to 60° C. and 85° C. for 30 minutes, cooled to 20° C. and the  $\text{CaCl}_2$  solution added. Table 1, Exp. E shows the coagulation data of the above typical arsenate "milk"; no significant hysteresis was observed on holding subsequent to the heat treatments.

Two unsuccessful attempts were made to prepare a colloidal calcium arsenate sol in gelatin by using calcium succate and M/10  $\text{H AsO}_4$  solution. A heavy precipitate is first formed but as the addition of acid increases and the pH of the mixture approaches that of natural milk the precipitate disappears and at the pH 6.67 the solutions are nearly transparent. Evidently a colloidal calcium arsenate does not exist at the pH of natural milk. This undoubtedly explains why the  $\text{CaCl}_2$  requirements of the calcium caseinate-arsenate "milks" were similar to those of the pure calcium caseinate sols.

Marui found that when sufficient  $\text{CaCl}_2$  was added to a solution of casein in sodium acetate-oxalate it became milky and would coagulate with rennet. We extended this observation to determine whether or not a complex of calcium caseinate and colloidal calcium oxalate would exhibit a hysteresis when heated and subsequently coagulated with rennet at 40° C. These "milks" were prepared in the general manner already described. A typical example is as follows: 30 gm. casein, 1020 cc. limewater and 95 cc. M/10 oxalic acid slowly added with the aid of mechanical stirring; pH after dilution to 1200 cc. was 6.84.

These systems, like the arsenate "milks" and the caseinate sols, required more  $\text{CaCl}_2$  than the caseinate-phosphate complexes; in all cases it was

added after heating. In order to coagulate the above "milk" in the desired time one cc. 0.35N  $\text{CaCl}_2$  solution was required for each 50 cc. Table 1, Exp. F shows the coagulation times of the calcium caseinate-collodial calcium oxalate "milk" described above after heating to 60° C. and 85° C. for 30 minutes. No hysteresis is apparent, the heating being without effect on the rate of coagulation.

CATAPHORESIS OF HEATED SOLS OF CALCIUM CASEINATE AND  
CALCIUM CASEINATE-CALCIUM PHOSPHATE "MILK"<sup>4</sup>

Richardson and Palmer (17) have shown that slightly acid calcium caseinate sols exhibit an increased rate of migration in an electric field after heating in boiling water. It was hoped that the hysteresis-like phenomenon might also be followed by cataphoretic measurements and preliminary observations were made by the Northrup-Kunitz method of electrophoresis. The details of this method are discussed by Moyer (18) and will not be included here. With this method the individual particles are observed in a dark field and the average of 10 observations at both "stationary levels" are used for the calculation of the velocity of migration. The velocities are expressed in  $\mu/\text{sec.}/\text{volt}/\text{cm.}$

The great dilution of the sol necessary for observation fosters a peptization of the particles, especially if a sodium buffer is used. Therefore, a calcium buffer was prepared by adding clear limewater to dilute  $\text{H}_3\text{PO}_4$  solution until a stable pH of 6.67 was reached. The clear liquid was carefully siphoned off and supercentrifuged at 40,000 R.P.M. Only a very few particles were visible in the ultramicroscope.

A 5 per cent calcium caseinate sol was prepared by triturating 2.5 gm. casein in 45 cc. limewater and diluting to 50 cc. A calcium caseinate-phosphate complex was prepared with 2.5 gm. casein, 93 cc. limewater and 9 cc. 0.6 per cent  $\text{H}_3\text{PO}_4$  solution; the resulting pH was 6.61. Both sols were aged 14 hours at 5° C. and then lightly centrifuged before using. The calcium caseinate sol was heated to 85° C. for 30 minutes while portions of the caseinate-phosphate complex were heated to 60° C. and 85° C. for 30 minutes and then cooled immediately to 20° C. The velocity of the particles was measured at 10, 40, and 70 minutes after heating. Table 2 shows that the effects of heating on the velocity of migration in an electric field are more pronounced on the calcium caseinate-phosphate complex than on the calcium caseinate sol.

DISCUSSION

From the experimental findings it is apparent that those components in milk which are involved in the clotting phenomenon in the presence of

<sup>4</sup> Direction and assistance in this phase of the work was rendered by Dr. L. S. Moyer, National Research Council Fellow in the Division of Agricultural Biochemistry, University of Minnesota.

TABLE 2

*Effects of heating a calcium caseinate sol and a calcium caseinate phosphate "milk" on their migration in an electric field*

TYPE OF SYSTEM	HEATING TEMPERATURE	VELOCITY OF MIGRATION IN R/SEC /VOLT/CM. AT VARIOUS PERIODS AFTER HEATING			
		0	10 <sup>1</sup>	10	70 min.
Calcium Caseinate	Unheated	1.42			
or	85		1.54	1.46	
Calcium Caseinate	Unheated	1.36			
	60		1.72	1.70	1.63
Phosphate	85		1.72	1.72	1.74

<sup>1</sup> Approximately 10 minutes were required to cool and prepare the first samples for observation.

rennet are likewise responsible for the hysteresis-like effect exhibited by heated milks when coagulated at definite time intervals after heating. Using coagulation as a criterion, since no special effort was made to secure clots, it was found that heated artificial systems of calcium caseinate-colloidal calcium phosphate show a progressive loss of coagulability with age.

It is the opinion of György (19), Kometiani (20) and Pyne (21) that the calcium caseinate and colloidal calcium phosphate systems in natural milk exist in a chemical union. If this opinion is correct a like association must exist to a certain extent in the artificial complex. Nevertheless, our results have shown that such a union is not essential either for coagulation by rennet or for the accompanying hysteresis, inasmuch as equal portions of the separate sols of calcium caseinate and colloidal calcium phosphate when mixed before heating produced a coagulation and hysteresis similar to that of the complex. The artificial systems even exhibited some evidence of the accelerating effect of low temperature heating on rennet coagulability which has been observed for natural milk.

The effects of heat upon the calcium caseinate fraction appear much less significant than previously suggested. Although ultramicroscopic observations revealed some slight physical change toward a coarser colloidal dispersion this effect was not registered by coagulability or hysteresis. Even boiling an artificial caseinate-phosphate "milk" failed to impair the coagulability of new "milks" prepared with casein isolated from the heated complex. Likewise when the colloidal calcium phosphate fraction was heated before mixing with the caseinate the effects of delayed addition of rennet were *nil* although minor differences in heating temperature were evident. The presence of both the caseinate and phosphate during the heating process is essential for the hysteresis-like phenomenon.

The decrease in pH of the heated phosphate sols is greatly masked by the presence of caseinate when the two sols are mixed in equal portions. This is true even to a greater degree when both fractions are present throughout the heat-treatment. Such extreme changes in hydrogen-ion concentration might mitigate other effects of heat such as a loss of much colloidal phosphate material. The peptization of colloidal calcium phosphate observed is contrary to the existing beliefs of the effects of heat on the calcium salts in natural milk.

It is not to be inferred that the mere presence of a second colloid is the criterion for the hysteresis behavior in the heated systems. We produced coagulation in a calcium caseinate-colloidal calcium oxalate "milk" as did Marui (14), but these complexes showed no hysteresis-like behavior after heating. It appears that colloidal calcium phosphates are essential for this phenomenon.

The preliminary cataphoretic studies of the heated calcium caseinate-phosphate complex and the heated caseinate sol indicate that the hysteresis phenomenon cannot be followed by changes in the velocity of migration of the colloidal particles. The effects observed seem to be somewhat contrary to those obtained by Richardson and Palmer (17) when using a modified Burton cataphoretic tube with raw and heated calcium caseinate sols. However, the experimental conditions of the present investigation are not strictly comparable to those of the former so that final judgement should be reserved. Nevertheless, it is indicated here that the effects of heat treatment on the cataphoretic velocity of rennet coagulable sols are not so closely related to their coagulability as the experiments of Richardson and Palmer suggested.

Our experiments cannot be interpreted as disproving the belief of Moir (4) regarding the rôle of a coagulation of lactalbumin in natural milk in connection with the phenomenon investigated. However, they suggest that Moir's findings are not of major importance in explaining it for natural milk.

#### CONCLUSIONS

1. Heated calcium caseinate-colloidal calcium phosphate "milks" as well as mixtures of equal parts of the two separate sols exhibit a hysteresis-like behavior when coagulated with rennet at definite intervals after heating.
2. Heat-treatment of calcium caseinate sols is practically without effect on their rate of coagulation either alone or when mixed with colloidal calcium phosphate.
3. Heat-treatment of the colloidal calcium phosphate portion before adding the caseinate is practically without effect on the rate of coagulation of the mixture.

4. The hysteresis effects of heat on the complex result from the presence of both the caseinate and phosphate during heat-treatment.
5. A complex containing colloidal calcium oxalate exhibits no hysteresis as a result of heating.
6. A colloidal calcium arsenate does not exist at the pH of natural milk.
7. Cataphoretic studies do not appear applicable as a means of following the hysteresis; additional work should be done, however, before discarding it. The fact that heating caused a greater increase in velocity of migration of a caseinate-phosphate complex than a calcium caseinate sol shows that the effects of heat are greatest in the presence of the colloidal phosphates.

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# American Dairy Science Association Announcements

## COMMITTEES OF THE MANUFACTURING SECTION OF THE AMERICAN DAIRY SCIENCE ASSOCIATION, 1935

### *Committee on National Intercollegiate Dairy Products Judging Contest:*

H. W. Gregory (Purdue), Chairman  
R. B. Stoltz (Ohio)  
Wm. White (U. S. D. A.)  
P. A. Downs (Nebraska)  
R. W. Smith, Jr. (Vermont)

### *Committee on Chemical Methods for the Analysis of Milk and Dairy Products:*

E. S. Guthrie (Cornell), Chairman  
H. A. Ruehe (Illinois)  
L. C. Thomsen (Wisconsin)  
G. H. Wilster (Oregon)  
W. H. Martin (Kansas)  
W. D. Swope (Pennsylvania)  
F. J. Doan (Pennsylvania)  
B. L. Herrington (Cornell)

### *Subcommittee on Milk and Cream:*

H. A. Ruehe (Illinois), Chairman  
R. W. Bell (U. S. D. A.)  
E. W. Bird (Iowa)

### *Subcommittee on Butter:*

L. C. Thomsen (Wisconsin), Chairman  
D. H. Nelson (California)  
S. T. Coulter (Minnesota)  
G. H. Wilster (Oregon)

### *Subcommittee on Cheese:*

G. H. Wilster (Oregon), chairman  
W. V. Price (Wisconsin)  
A. J. Morris (Utah)  
E. F. Goss (Iowa)

*Subcommittee on Ice Cream:*

W. H. Martin (Kansas), Chairman  
W. E. Peterson (Minnesota)  
L. K. Crowe (Nebraska)  
P. S. Lucas (Michigan)

*Subcommittee on Condensed and Evaporated Milk:*

W. D. Swope (Pennsylvania), Chairman  
E. O. Anderson (Connecticut)  
L. M. Thurston (West Virginia)  
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*Subcommittee on Dry Milk:*

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J. L. Hileman (Dairymen's League, Syracuse, N. Y.)  
G. C. Supplee (Dry Milk Co., Bainbridge, N. Y.)  
R. W. Titus (Nestle's Milk Products Co., Marysville, Ohio)  
J. I. Keith (Oklahoma)

*Subcommittee on Skimmilk, Buttermilk, and Whey:*

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B. E. Horrall (Purdue)  
E. W. Bird (Iowa)  
A. H. Johnson (National Dairy Products Corp., Baltimore, Md.)

*Committee on Bacteriological Methods for the Analysis of Milk and Dairy Products:*

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E. G. Hood (Ottawa, Canada)  
A. C. Fay (Kansas)  
P. A. Downs (Nebraska)  
P. S. Prickett (Mead, Johnson & Co., Evansville, Ind.)  
R. S. Breed (Geneva, N. Y.)

*Subcommittee on Butter:*

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B. W. Hammer (Iowa)  
E. G. Hood (Ottawa, Canada)  
R. P. Myers (National Dairy Products Corp., Baltimore, Md.)

*Subcommittee on Cheese:*

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W. C. Frazier (Wisconsin)

W. S. Golding (Idaho)  
W. V. Price (Wisconsin)  
C. D. Kelly (Old Tavern Farms, Inc., Portland, Me.)

*Subcommittee on Ice Cream:*

A. C. Fay (Kansas), Chairman  
B. W. Hammer (Iowa)  
F. W. Fabian (Michigan)

*Subcommittee on Condensed and Evaporated Milk:*

P. A. Downs (Nebraska), Chairman  
B. W. Hammer (Iowa)  
W. A. Cordes (Blue Valley Creamery Co., Chicago)

*Subcommittee on Dry Milk:*

P. S. Prickett (Mead, Johnson & Co., Evansville, Ind.), Chairman  
G. C. Supplee (Dry Milk Co., Bainbridge, N. Y.)  
R. Schneiter (U. S. D. A.)

*Subcommittee on Market Milk:*

R. S. Breed (Geneva, N. Y.), Chairman  
J. A. Anderson (New Jersey)  
H. E. Bremer (Montpelier, Vt.)  
M. W. Yale (Geneva, N. Y.)  
C. K. Johns (Ottawa, Canada)  
C. N. Stark (Cornell)



# AMERICAN DAIRY SCIENCE ASSOCIATION

## *The Thirtieth Annual Meeting*

St. Paul, Minnesota, June 24 to 27, 1935

### GENERAL PROGRAM

#### *Monday, June 24*

- 9 A. M.—5 P. M.—General registration and room registration.  
Haecker Hall, University Farm.
- 1:30 P. M.—4:30 P. M.—Extension Section, Haecker Hall, Room 210.
- 2 P. M.—Dairy products judging and special butter judging  
demonstration by federal butter graders.  
Haecker Hall, Creamery Room
- 3 P. M.—Executive Committee Meeting, Haecker Hall, Room  
214.
- 5 P. M.—9 P. M.—General registration and room registration.  
Women's Dormitory, University Farm.
- 7:30 P. M.—Opening Session and Business Meeting.  
Auditorium, Administration Building, University  
Farm.  
President C. L. Roadhouse, presiding.  
Address of Welcome—President L. D. Coffman,  
University of Minnesota.  
Address of Welcome—Dean W. C. Coffey, Uni-  
versity of Minnesota.  
*Business Meeting.*
- 8:30 P. M.—Informal social get-together for members and guests  
and their families, Home Economics Building,  
University Farm.

#### *Tuesday, June 25*

- 8 A. M.—4 P. M.—General registration and room registration.  
Haecker Hall, University Farm.
- 9 A. M.—12 NOON—Joint Meeting of Section O, American Association  
for the Advancement of Science, American Dairy  
Science Association, Corn Belt Section of the  
American Society of Agronomy, American Society  
of Horticultural Science, Great Plains Section,  
American Phytopathological Society, The Genetics  
Society of America, and The American Society of  
Plant Physiologists. Auditorium, Administration  
Building, University Farm.  
Symposium: Improving the Germ Plasm of  
Domestic Plants and Animals, H. K. Hayes,  
Chairman, Section O, Vice President, A. A.  
A. S., presiding.

Introductory Address: Dean W. C. Coffey, University of Minnesota.

Some Accomplishments with Animals: Dr. J. L. Lush, Iowa State College.

Analysis and Synthesis in the Development of New Varieties of Plants: Dr. O. S. Aamodt, University of Wisconsin.

12 NOON-1 P. M.—Lunch hour.

1 P. M.-2 P. M.—Extension Exhibits, Haecker Hall, Room 101.

1 P. M.-4 P. M.—Production Section, Haecker Hall, Room 100.

1 P. M.-4 P. M.—Manufacturing Section, Haecker Hall, Room 109.

4 P. M.-4:30 P. M.—Section Committee Meetings.

4 P. M.-4:30 P. M.—Members, guests and their families leave for trip to Land-O-Lakes Creameries, Inc., Minneapolis.

6 P. M.—Complimentary dinner and entertainment as guests of Land-O-Lakes Creameries, Inc. Admission by ticket.

### *Wednesday, June 26*

9 A. M.-12 NOON—Production Section. Haecker Hall, Room 100.

9 A. M.-12 NOON—Manufacturing Section. Haecker Hall, Room 109.

12 NOON-1 P. M.—Complimentary Dairy Lunch. Haecker Hall.

1 P. M.-2 P. M.—Production Section Business Meeting. Haecker Hall, Room 100.

1 P. M.-2 P. M.—Manufacturing Section Business Meeting. Haecker Hall, Room 109.

1 P. M.-2 P. M.—Extension Section Business Meeting. Haecker Hall, Room 210.

2 P. M.-4:30 P. M.—Section on Problems and Methods of Instruction. Haecker Hall, Room 100.

2 P. M.-4:30 P. M.—Manufacturing Section. Haecker Hall, Room 109.

2 P. M.-4:30 P. M.—Extension Section. Haecker Hall, Room 210.

5 P. M.—Leave for Minneapolis Automobile Club.

7 P. M.—Subscription banquet and entertainment at Minneapolis Automobile Club. (Tickets to be purchased on registration.)

### *Thursday, June 27*

9 A. M.-10 A. M.—Closing general session and business meeting. Auditorium, Administration Building, University Farm. President C. L. Roadhouse presiding.

10 A. M.-1 P. M.—Production Section. Haecker Hall, Room 100.

10 A. M.-1 P. M.—Manufacturing Section. Haecker Hall, Room 109.

1 P. M.-2 P. M.—Lunch hour.

2 P. M.—Leave for arranged tours to cooperative and independent dairy plants in the Twin Cities and trips to farms of various breeders in the vicinity of the Twin Cities. Information regarding these tours and trips will be available on registration. Members desiring any given tour or trip will register for same at time of general registration.

## SECTION PROGRAMS

## EXTENSION SECTION

Monday afternoon, June 24, 1:30-4:30 o'clock

Haecker Hall, Room 210

FLOYD JOHNSTON, *Chairman*

COW TESTING (A. B. Nystrom, Chairman) FEEDING (C. L. Blackman, Chairman), CALF CLUB (D. M. Seath, Chairman).

(Papers limited to 10 minutes)

- E1—Revision of record forms. J. W. Linn, Kansas State College.
- E2—Testers conferences—Official herd testing and testers organizations. W. T. Crandall, Cornell University.
- E3—Farm account week through D. H. T. A. and bi-monthly testing. A. J. Cramer, University of Wisconsin.
- E4—Analysis of D. H. I. A. data. G. A. Williams, Purdue University.
- E5—A yearly association program. A. B. Nystrom, Bureau of Dairy Industry, U. S. D. A.
- E6—Developing a pasture program. L. A. Higgins, Mississippi A. and M. College.
- E7—A feeding program based on D. H. I. A. reports. J. O. Tretsven, Montana State College.
- E8—County feeding programs. H. R. Searles, University of Minnesota.
- E9—The five session feeding schools. C. L. Blackman, University of Ohio.
- E10—Record keeping and reporting—Dairy club work. R. G. Connelly, Virginia A. and M. College.
- E11—Basis for premium awards in dairy calf club work. Ivan McKellip, University of Ohio.
- E12—Making dairymen out of club members. D. M. Seath, Kansas State College.

## PRODUCTION SECTION

Tuesday afternoon, June 25, 1:00-4:30 o'clock

Haecker Hall, Room 100

C. Y. CANNON, *Chairman*

MILK SECRETION, HORMONES, GENETICS, VITAMINS

(Papers limited to 15 minutes)

- P1—The involution of the mammary gland. E. P. Reineke and C. W. Turner, University of Missouri.
- P2—The ovary stimulating interaction of blood serum from cattle and gonadotrophic extracts. L. E. Casida, University of Wisconsin.
- P3—A review of color inheritance in dairy cattle. Heman L. Ibsen and H. W. Cave, Kansas State College.

- P4—The dietary requirements of goats for vitamin E (A progress report). John I. Wilson, B. H. Thomas and C. Y. Cannon, Iowa State College.
- P5—A preliminary study of the vitamin D requirement of calves when fed natural milk as the source of this vitamin. C. F. Huffman and C. W. Duncan, Michigan State College.
- P6—The pathology of rickets in dairy calves. H. Ernest Bechtel, E. T. Hollman and C. F. Huffman, Michigan State College.
- P7—The color and carotene content of various home-grown roughage rations and the influence of these rations on the color, carotene and vitamin A potency of the butterfat. R. E. Hodgson, J. C. Knott, H. K. Murer and R. R. Graves, Bureau of Dairy Industry, U. S. D. A., and Washington State College.
- P8—The vitamin A activity of butter produced by cows fed alfalfa hay and soybean hay cut in different stages of maturity. J. H. Hilton, J. W. Wilbur and S. M. Hauge, Purdue University.
- P9—"Caratone" in rations for dairy calves. A. H. Kuhlman, W. D. Gallup and Earl Weaver, Oklahoma A. and M. College.
- P10—Blindness in cattle of nutritional origin associated with constriction of the optic nerve. L. A. Moore, C. F. Huffman, and C. W. Duncan, Michigan State College.
- 4:00 P. M. Committee meetings.

## MANUFACTURING SECTION

Tuesday afternoon, June 25, 1935 10:00–4:30 o'clock

Haecker Hall, Room 109

M. J. MACK, *Chairman*

### CHEMISTRY

(Papers limited to 10 minutes)

- M1—Studies on the ash and nitrogen distribution of processed cheese as affected by the salts used and a comparison of the methods used for the determination of the pH of the cheese. Hugh L. Templeton, University of Wisconsin.
- M2—Effects of some ions on the properties of ice cream mixes. J. I. Keith, C. W. Rink and Earl Weaver, Oklahoma A. and M. College.
- M3—The hydrogen ion concentration and titratable acidity of butter, cream and buttermilk. O. F. Hunziker and W. A. Cordes, Blue Valley Creamery Co., Chicago.
- M4—Range of hydrogen ion concentration in sour cream butter. E. H. Parfitt, Purdue University.
- M5—The thiocyanogen value as a means of measuring unsaturated fatty acids in butterfat. O. J. Hill and L. S. Palmer, University of Minnesota.
- M6—The effect of homogenization on some of the fat constants of milk. I. A. Gould and G. M. Trout, Michigan State College.

- M7—Variations in physical properties of milk. G. P. Sanders, K. I. Matheson and I. A. Burkey, Bureau of Dairy Industry, U. S. D. A.
- M8—Soft curd character induced in milk by intense sonic vibration. Leslie A. Chambers, University of Pennsylvania.
- M9—Variations in the curd tension of the milk throughout the complete lactation period. M. H. Berry, University of Maryland.
- M10—Effect of mastitis upon milk quality and composition. P. A. Downs, University of Nebraska.
- M11—The relation of mastitis to the rennet coagulability and curd strength of milk. H. H. Sommers and Helene Matson, University of Wisconsin.
- M12—Structural changes occurring in casein as shown by X-ray diffraction studies. S. L. Tuckey, H. A. Ruehe and G. L. Clark, University of Illinois.
- 4:00 P. M.—Committee Meetings.

### EXTENSION SECTION

Tuesday afternoon, June 25, 1:00–2:00 o'clock

Haecker Hall, Room 101

FLOYD JOHNSTON, *Chairman*

#### EXHIBITS

Extension exhibition

Earl N. Schultz, Chairman, Iowa State College,  
Ramer Leighton, University of Minnesota,  
J. E. Crosby, Jr., University of Missouri.

The dairy extension exhibits from the states will be on display throughout the entire meeting. They will be discussed by the above committee during this hour. Those especially interested may extend this time.

### MANUFACTURING SECTION

Wednesday morning, June 26, 9:00–12:00 o'clock

Haecker Hall, Room 109

M. J. Mack, *Chairman*

#### CHEMISTRY AND TECHNOLOGY

(Papers limited to 10 minutes)

- M13—Effects of time and temperature of holding milk heat-treated at various temperatures on its subsequent coagulation by rennet. Milton E. Powell, University of Minnesota.
- M14—Determinations of copper in sugar condensed milk and some relations between the copper content and off flavor in strawberry ice cream.

Harold L. Link, Harry J. Konen and L. A. Baumann, Xavier University and French-Bauer, Inc., Cincinnati.

- M15—The application of the Minnesota Babcock method to the testing of ice cream, concentrated milk and chocolate milk. L. M. Thurston and W. Carson Brown, West Virginia University.
- M16—The standardization of the Borden Body Flow-Meter for determining the apparent viscosity of cream. J. C. Hening, New York Agricultural Experiment Station, Geneva.
- M17—Judging sweet cream. J. H. Nair, D. E. Mook and R. S. Fleming, Borden's Research Laboratory, Syracuse.
- M18—Some factors affecting the properties of whipped cream. W. S. Mueller, M. J. Mack and H. G. Lindquist, Massachusetts State College.
- M19—Frequency of the flavor defects in milk. Earl Weaver, E. L. Fouts and P. C. McGilliard, Oklahoma A. and M. College.
- M20—Effects of feeds on oxidized flavors in pasteurized milk. Ed. Prewitt and E. H. Parfitt, Purdue University.
- M21—Methods of studying feed effects on the physical properties of butterfat. Willis D. Gallup, J. I. Keith and A. H. Kuhlman, Oklahoma A. and M. College.
- M22—Effect of a heavy cottonseed meal ration on milk and butter. J. I. Keith, A. H. Kuhlman, Earl Weaver and Willis D. Gallup, Oklahoma A. and M. College.

## PRODUCTION SECTION

Wednesday morning, June 26, 9; 00–12: 00 o'clock

Haecker Hall, Room 100

C. Y. CANNON, *Chairman*

VITAMINS (continued), SILAGE, HAY  
(Papers limited to 15 minutes)

- P11—A study of the malnutrition incident to the heavy feeding of cottonseed meal. S. I. Bechdel and S. R. Skaggs, Pennsylvania State College.
- P12—Influence of the ration on the vitamin C content of milk. W. H. Ridell, C. H. Whitnah and J. S. Hughes, Kansas State College.
- P13—Effect of the condition of corn plant at cutting upon the carotene content of silage. E. A. Kane and C. A. Cary, Bureau of Dairy Industry, U. S. D. A.
- P14—The stack silage method of preserving forage crops and the comparative nutritive value of oat and pea silage made in a stack and in a tower. J. C. Knott, R. E. Hodgson and R. R. Graves, Washington State College and Bureau of Dairy Industry, U. S. D. A.
- P15—Pea vine silage as a feed for dairy cattle. J. C. Tretsven, Montana State College.
- P16—Studies regarding the use of mineral acids for the preservation of forage. C. C. Hayden, A. E. Perkins, C. F. Monroe, W. E. Krauss,

- C. E. Knoop, R. G. Washburn and T. S. Sutton, Ohio Agricultural Experiment Station, Wooster.
- P17—Molasses as a preserving agent in making soybean silage. E. C. Elting, Clemson College, South Carolina.
- P18—Experimental cottonseed meal ration plus silage versus herd ration, hay and silage feeding. R. H. Lush, Louisiana State University.
- P19—The digestibility and feeding value of Russian thistle hay. H. W. Cave, W. H. Riddell and J. S. Hughes, Kansas State College.
- P20—*Lespedeza Sericca* feeding trials with dairy cows. C. E. Wylie and S. A. Hinton, University of Tennessee.

## MANUFACTURING SECTION

Wednesday afternoon, June 26, 1:00-4:30 o'clock

Haecker Hall, Room 109

C. J. Mack, *Chairman*

### BACTERIOLOGY

(Papers limited to 10 minutes)

1 P. M.-2 P. M.—Section business meeting.

- M23—The detection and control of bovine mastitis. G. J. Hucker, New York Agricultural Experiment Station, Geneva.
- M24—Studies on aseptically drawn milk from Bang's disease positive and Bang's disease negative cows. H. B. Morrison and F. E. Hull, University of Kentucky.
- M25—Bitter flavor in cheddar cheese from pasteurized milk. C. A. Phillips, University of California.
- M26—Study of a gassy defect in cream cheese. W. J. Corbett, W. C. Frazier and W. V. Price, University of Wisconsin.
- M27—Varieties of the genus *Oospora* found in cream and butter. C. M. Sorensen, Purdue University.
- M28—The disappearance of acetylmethylcarbinol and diacetyl in butter cultures. G. L. Stahly, M. B. Michaelian, C. H. Werkman and B. W. Hammer, Iowa State College.
- M29—A study of *Escherichia-aerobacter* organisms in pasteurized milk. Joseph L. Minkin and L. H. Burgwald, Ohio State University.
- M30—Observations on yeasts causing gas in sweetened condensed milk. H. C. Olson and B. W. Hammer, Iowa State College.
- M31—Standard laboratory methods for the control of dairy products. Robert S. Breed, New York Agricultural Experiment Station, Geneva.

## EXTENSION SECTION

Wednesday afternoon, June 26, 1:00-4:30 o'clock

Haecker Hall, Room 210

FLOYD JOHNSTON, *Chairman*

SIRES (J. G. KENDRICK, *Chairman*) QUALITY IMPROVEMENT

(A. C. BOLTZER, *Chairman*)

(Papers limited to 10 minutes)

1 P. M.—2 P. M.—Section business meeting.

E13—Listing D. H. I. A. proved sires—Permanent records. E. N. Schultz, Iowa State College.

E14—Terminology for indicating the merits of D. H. I. A. sires. S. J. Brownell, Cornell University.

E15—Analysis of D. H. I. A. proved sire records. E. J. Perry, State University of New Jersey.

E16—Sons of D. H. I. A. proved sires. D. H. Fourn, University of Idaho.

E17—Standardized lactation records for reporting dam and daughter comparisons in D. H. I. A. J. F. Kendrick, Bureau of Dairy Industry, U. S. D. A.

E18—Quality improvement committee report. E. C. Scheidenhelm, University of Nebraska.

E19—Quality improvement committee report. C. A. Hutton, University of Tennessee.

E20—Quality improvement committee report. Fred H. Abbott, University of California.

E21—Quality improvement committee report. A. J. Mann, Connecticut Agricultural College.

E22—Quality improvement committee report. A. C. Baltzer, Michigan State College.

## SECTION ON PROBLEMS AND METHODS OF INSTRUCTION

Wednesday afternoon, June 26, 2:00-4:30 o'clock

Haecker Hall, Room 100

EARL WEAVER, *Chairman*

(Papers limited to 15 minutes)

I1—A basic curriculum for an agricultural college. H. P. Davis, University of Nebraska.

I2—Certain suggestions for a course of study for those majoring in dairy industry. J. H. Frandsen, Massachusetts State College.

I3—Building a course in dairy husbandry. H. P. Davis, University of Nebraska.

I4—Frequent quizzes in teaching dairy elements. E. L. Fouts and J. I. Keith, Oklahoma A. and M. College.

I5—Adjusting dairy instruction to the needs of the state. C. E. Wylie, University of Tennessee.

- I6—The integrated course of study in agriculture. A. M. Field (by invitation) Division of Agricultural Education, University of Minnesota.

## PRODUCTION SECTION

Thursday morning, June 27, 10:00–1:00 o'clock

Haecker Hall, Room 100

C. Y. CANNON, *Chairman*

### NUTRITION, RATIONS, PERFORMANCE

(Papers limited to 15 minutes)

- P21—The effect of soybeans on the fat content of milk. J. W. Wilbur, J. H. Hilton and co-workers, Purdue University.
- P22—The effect of quality and level of protein intake on growth and milk production. I. W. Rupel, G. Bohstedt and E. B. Hart, University of Wisconsin.
- P23—Milk and butterfat production on high and low protein rations. C. A. Cary, Bureau of Dairy Industry, U. S. D. A.
- P24—Extremes in protein feeding—Bearing of results on the protein-feeding standard. A. E. Perkins, Ohio Agricultural Experiment Station.
- P25—Changes in weight of new born calves as related to the method of feeding. C. L. Cole, University of Minnesota.
- P26—The nutrients required by dairy cows kept in an open shed versus cows kept in a dairy barn. J. R. Dice, North Dakota Agricultural College.
- P27—Formulae for calculating rations for milk cows. A. H. Kuhlman, Oklahoma A. and M. College.
- P28—Permanent records in the station herd. R. B. Becker, University of Florida.
- P29—Effect of breeding efficiency and culling on herd production. Lynn Copeland, American Jersey Cattle Club, New York City.

## MANUFACTURING SECTION

Thursday morning, June 27, 10:00–1:00 o'clock

Haecker Hall, Room 109

C. J. MACK, *Chairman*

### CHEESE, BUTTER AND TECHNOLOGY

(Papers limited to 10 minutes)

- M32—Acidity in the manufacture of cream cheese. Z. D. Roundy and W. V. Price, University of Wisconsin.
- M33—The manufacture of a soft cheese of the Bel Paese type. Robert R. Farrar, Bureau of Dairy Industry, U. S. D. A.

- M34—Experiments with canned cheddar cheese. E. L. Reichart, University of Nebraska.
- M35—The vitamin A content of sour cream butter, sweet cream butter and margarine. I. L. Hathaway and H. P. Davis, University of Nebraska.
- M36—Technic, examination and reporting extraneous matter in butter. B. E. Horrall, Purdue University.
- M37—Notes on the national cream quality improvement campaign. M. E. Parker, Seal-test System Laboratories, Inc., Cleveland.
- M38—Efficiency of electrically operated tanks versus ice in the cooling of milk. J. H. Frandsen, Massachusetts State College.
- M39—Frozen brines as refrigerants of ice cream in cabinets and shipping containers. H. H. Sommer, University of Wisconsin.
- M40—An experimental ice cream freezing unit. J. I. Keith and C. W. Rink, Oklahoma A. and M. College.
- M41—Methods for testing condensing pans. L. C. Thomsen, University of Wisconsin.

# JOURNAL OF DAIRY SCIENCE

VOLUME XVIII

JULY, 1935

NUMBER 7

## ABSTRACTS OF PAPERS PRESENTED AT ANNUAL MEETING

### PRODUCTION SECTION

*P1. The involution of the mammary gland.* E. P. REINEKE AND C. W. TURNER, University of Missouri.

As a result of recent work, the part played by the hormones of the ovary and the anterior pituitary in the regulation of the growth and functional activity of the mammary gland has become established. It is generally agreed that the growth of the duct system is stimulated by the estrogenic hormone. The growth of the lobule-alveolar system is regulated by a hormone secreted by the corpus luteum, acting in conjunction with the estrogenic hormone. The stimulus to secretory activity comes from a hormone secreted by the anterior lobe of the anterior pituitary, a small structure located at the base of the brain.

Following weaning and the cessation of milk secretion, it has been observed in a series of laboratory animals that the lobule-alveolar structure gradually degenerates, and the gland returns to a duct system similar to that found in a non-parous animal. Before heavy lactation can be reinduced in such animals, the lobule-alveolar system must be regrown. This observation is of considerable interest in connection with its relation to the rate of decline of lactation in normal animals. It is also of importance in considering the possibility of the experimental stimulation of lactation or of restoring milk secretion in cows that have been dry for some time.

The object of the present report is to present the results of a study of the histological picture of the mammary glands of a series of goats, taken at various intervals following the cessation of milking.

There is at first an accumulation of milk in the gland, followed by resorption of the milk and progressive atrophy of the lobule-alveolar system. The alveoli shrink in size, and eventually collapse, finally disappearing completely. In early stages there is a rapid infiltration of leucocytes into the gland parenchyma and the surrounding connective tissue.

<sup>1</sup> Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station. Journal Series No. 405.

In the completely involuted gland only the large ducts and lateral branches, surrounded by connective tissue, remain

Comparisons made between involutionary stages and various developmental stages of pregnancy, emphasize the fact that the mammary gland undergoes a complete involution during the drying up period, and must be rejuvenated by the hormones of pregnancy before the next lactation. It is recognized, however, that when pregnancy and lactation are concurrent, the status quo of the mammary gland is probably maintained.

That these observations made on goats are also true of dairy cows is indicated by a preliminary study of the mammary glands of cows taken at various stages of lactation. Here it was observed that some of the lobules involute while others are still actively secreting milk. This suggests the necessity of regrowing these involuted portions in the case of cows at advanced stages of lactation before an appreciable increase in milk production can be secured by stimulation with the lactogenic hormone

*P2. The ovary-stimulating interaction of blood serum from cattle and gonadotrophic extracts.* L. E. CASIDA, University of Wisconsin.

Blood serum from cattle interacts with unfractionated gonadotrophic extracts of the anterior pituitary to augment the weights of ovaries of test rats over that produced by the pituitary extract alone. Such interaction is not obtained between cow blood serum and gonad stimulating extracts of urine of pregnant women or blood serum from pregnant mares. Neither is such interaction obtained between cow serum and *purified* follicle-stimulating or luteinizing extracts of the anterior pituitary. When, however, follicle-stimulating and luteinizing fractions are used together, there is augmentation of their combined effect by addition of cow serum.

There is no apparent correlation between the stages of the reproductive cycle represented by the donor cattle and the augmenting activity of their blood serum.

The augmenting action of the serum is slightly effective when administered orally and is not injured by boiling.

Interaction of blood serum with unfractionated pituitary extracts may account for some of the differences in the reactions of ovaries of heifer calves to unfractionated pituitary extracts and to pregnant mare blood serum.

*P3. A review of color inheritance in dairy cattle.* HEMAN L. IBSEN AND H. W. CAVE, Kansas State College.

Illustrations are given of the self (*S*) and the recessive white-spotting (*s*) characters in cattle. A number of different modifiers of *s* are then taken into consideration. Among these are *Lw* (little white), *Pl* (pig-

mented leg), *Wr* (white restricter) and *DI* (distal leg spot). White face in Holsteins and gray color in Jerseys are also discussed.

*P4. The dietary requirements of goats for vitamin E* (A progress report).

JOHN L. WILSON, B. H. THOMAS, AND C. Y. CANNON, Iowa State College.

Since the discovery of vitamin E and its effect upon rats some investigators and many practitioners and stockmen have assumed that farm animals require this vitamin for normal reproduction. This assumption has led to supplementing stock rations with products known to be high in vitamin E content. Furthermore, the common cattle feeds have been tested with rats to determine their vitamin E content with the inference that such knowledge might aid in correcting reproductive troubles in cattle.

There are no experiments reported in the literature in which it has been proved that vitamin E is essential to the normal reproduction of ruminants. As a preliminary experiment in an attempt to determine the dietary requirements of ruminants for vitamin E, six female goats were placed on a ration consisting of alfalfa hay and a grain mixture which had been treated with a solution of ferric chloride and ether to inactivate the vitamin E naturally occurring in the ration. The resulting vitamin-E-free ration was supplemented with cod liver oil and yeast, both of which had been proven previously in tests on rats to be lacking in vitamin E. Each batch of treated ration was checked with rats to determine its freedom from vitamin E.

The health and reproduction of the goats has been excellent while being fed the experimental ration. Approximately one year after being put on the vitamin-E-free ration five of the original six females gave birth to nine kids (eight alive and one dead). At the end of the second year on the experimental ration all six of the original goats gave birth to nine kids (five males and four females).

Among the kids born to the original goats at the end of the first year were three females. Two of these were bred and have given birth to four (three males and one female) healthy and vigorous kids. For reasons as yet unknown to us the third female grew subnormally and was recently killed for histological examination.

All of the kids born the second year to both the original goats and the two first generation females were sired by bucks that were born to the original goats. These young bucks had received only the vitamin-E-free experimental ration.

It appears from these experiments that goats may not require vitamin E in their rations. If they do, then their requirements for this factor are below those of the rats which were used to determine the absence of

vitamin E from the goat rations. There is a remote possibility that vitamin E is synthesized in the body of the goat.

*P5. A preliminary study of the vitamin D requirement of calves when fed natural milk as the source of this vitamin.* C. F. HUFFMAN AND C. W. DUNCAN, Michigan State College.

Eighteen calves were used in this investigation. Six calves were fed the basal rachitic grain mixture supplemented with skim milk. The remaining calves were fed different levels of whole milk as their source of vitamin D. Blood plasma calcium, magnesium, and inorganic phosphorus values were determined each week.

Most of the animals were slaughtered at about 160 days of age. Roentgenograms and silver nitrate stained sections of the costo-chondral junctions were made of some of the ribs of each animal. Ash analysis of the 8th right rib was also made.

The results indicate that about 0.35 to 0.45 U. S. P. units of vitamin D from winter milk per pound of body weight meet the vitamin D requirement for maintenance and growth from birth to five months of age.

*P6. The pathology of rickets in dairy calves.* H. ERNEST BECHTEL, E. T. HALLMAN, AND C. F. HUFFMAN, Michigan State College.

This study was based on 5 normal and 11 ricketic grade-Holstein calves selected from a group of more than 100 animals available for this work. The ages of the calves varied from 151 to 520 days at death. The period of duration of the disease varied from 38 to 212 days.

Low vitamin D rickets in dairy calves was characterized principally by changes in the bones. These changes were always preceded by decreased concentrations of calcium and/or inorganic phosphorus in the blood plasma. The costo-chondral junction at the ventral end of the rib was selected as the best index to ricketic changes in the skeleton. Mid-frontal sections of about 4 millimeters in thickness were taken from the last 3 inches of the ventral end of each rib and studied by comparison of roentgenograms, photographs of specimens stained in silver nitrate solution, and histological sections.

In the specimens studied, histological changes were confined largely to a relatively small portion of the bone at the costo-chondral junction. Retarded provisional calcification of the cartilage matrix appeared to be the fundamental change in rickets. However, the most conspicuous changes in microscopic study were irregular removal of cartilage by the embryonic marrow, and accumulation of excess osteoid tissue. The beading of ricketic ribs appeared to be caused by accumulations of osteoid tissue, but was exaggerated in gross appearance on the medial border of the rib by in-

creased curvature of the rib in a medial direction. Cupping of the ventral-epiphyseal end of the diaphysis was not a prominent feature in roentgenograms of the costo-chondral junctions of ricketic calves.

Growth was an important modifying factor in rickets. More severe rickets was associated with more rapid growth. Younger calves developed more florid rickets than did older calves under similar management.

Low plasma calcium rickets appeared in this study to be histologically identical with rickets in which there were decreased concentrations in the plasma of both calcium and inorganic phosphorus.

*P7. The color and carotene content of various home-grown roughage rations and the influence of these rations on the color, carotene and vitamin A potency of the butterfat.* R. E. HODGSON, J. C. KNOTT, H. K. MURER, AND R. R. GRAVES, Bureau of Dairy Industry, U. S. D. A., and Washington State College.

Experiments were conducted to study the relationships of the color and carotene content of various home-grown feeds and the color, carotene and vitamin A content of the butterfat produced by cows receiving sole rations of these feeds. Also a comparison has been made of the color and carotene content of butterfat and its vitamin A potency. Three groups of seven Holstein cows in varying stages of lactation were fed rations of native grown forages cured as hay and as silage. One group was maintained throughout the winter on a ration of native grown and cured hay containing a mixture of the common grasses and clovers. A second group was maintained on a ration of silage alone while the third group received both hay and silage. Three kinds of silage were used in these studies, namely, (1) silage made from perennial forages similar to and taken from the same fields as that which was made into hay. (2) Oat and pea silage made in a tower silo, and (3) oat and pea silage made in a stack.

The groups of cows had been receiving these rations at least three months before the tests were begun. Color and carotene determinations were conducted on representative samples of pasture, native grown and cured hay and each of the three kinds of silage. At the same time the various feeds were sampled for analysis, representative samples of butterfat produced by each of the three groups of cows were analyzed for color and carotene. Vitamin A determinations were made on butterfat produced by (1) cows receiving the hay rations; (2) cows receiving silage made from perennial grasses and clover; and (3) cows receiving a ration containing both hay and silage. The color measurements were made by the method described by Nickerson, carotene by a photoelectric scopometer, the pigments being fractioned by methods modified from Willstatter and Stoll and more recently by Miller, and the vitamin A bio-assays according to the Sherman method.

Butterfat produced from cows receiving native grown and cured hay

was low in both color and carotene content as compared with that produced by cows receiving pasture or silage as their ration. The color and the carotene of the butterfat produced by cows on silage alone was only slightly less than that produced when they were on pasture. Cows receiving a ration containing approximately half silage produced butterfat with almost as high a color and carotene content as those receiving an all silage ration.

The dry matter of the silages had a materially higher carotene content than the native grown and cured hay. Butterfat produced from cows receiving hay and silage contained 1.64 times as much vitamin A while that produced by cows on all silage rations was 1.66 times as much vitamin A as butterfat produced by cows on hay alone.

*P8. The vitamin A activity of butter produced by cows fed alfalfa hay and soybean hay cut in different stages of maturity.* J. H. HILTON, J. W. WILBUR, AND S. M. HAUGE, Purdue University.

In work previously reported at the Purdue Station it was found that artificially dried hay was superior in vitamin A activity to field cured hay. Early cut alfalfa hay was also found to have a higher vitamin A value than late cut hay.

It, therefore, seemed advisable to conduct some feeding trials with artificially dried and field cured legume hays, cut in different stages of maturity, to see what effect they might have on the vitamin A activity of butterfat. Since alfalfa hay, and soybean hay to a lesser extent, have been shown to be very effective in maintaining a high vitamin A value of butter, these two hays were selected for this work. Samples of each of these hays were cut in the early stages of maturity and in the late stages of maturity. Portions of each cutting of each hay were field cured and artificially dried. Biological assays for vitamin A have been made on each sample of hay and on the butterfat from cows fed these respective hays.

In general it was found that the artificially dried hays produced butters of higher vitamin A value than the field cured hays. Early cut hays were more effective than late cut hays in producing butters of high vitamin A activity.

*P9. Caratone in rations for dairy calves.* A. H. KUELMAN, W. D. GALLUP, AND EARL WEAVER, Oklahoma A. & M. College.

Calves, used in continuing the work begun with older cattle to study the rôle of vitamin A in cottonseed meal rations, offer a means for comparing the values of several carriers of this vitamin.

Fifteen young calves were kept under similar conditions during the period of vitamin depletion. They were confined in a well-lighted barn, received whole milk for thirty to forty days, skim milk to the age of six months, and the basal ration of beet pulp and cottonseed meal supplemented with bone meal.

In general, low blood calcium or phosphorus values preceded or coincided with the appearance of characteristic symptoms of vitamin A deficiency. Exposure to direct sunlight or addition of a vitamin D supplement returned blood calcium and phosphorus to normal levels in ten to fourteen days, but did not improve the appetite, external appearance, or gains in weight.

Cod liver oil or canned tomatoes and aerated cod liver oil added to the rations of affected animals brought about improvement, stimulated appetites and increased gains in weight. Five control animals receiving the basal ration plus 30 cc. of cod liver oil daily and kept in the barn consistently exceeded the gains of normal calves.

Caratone, a commercial product recently put on the market as a source of vitamin A for livestock and poultry, was given to seven calves depleted in their stores of vitamin A. When supplemented with either aerated cod liver oil or viosterol, responses similar to those produced by cod liver oil were obtained.

Preliminary results indicate that, when supplemented with vitamin D, 5 cc. of caratone are as effective as 30 cc. of cod liver oil or 1200 cc. of canned tomatoes plus 30 cc. aerated cod liver oil in supplying the deficiency in the basal ration.

*P10. Blindness in cattle of nutritional origin associated with constriction of the optic nerve.* L. A. MOORE, C. F. HUFFMAN, AND C. W. DUNCAN, Michigan State College.

Thirty cases of blindness have occurred which are different from the true vitamin A type of blindness. The blindness is observed in calves following birth and in young growing dairy animals when a ration containing poor quality roughage has been fed. It is frequently associated with paralysis, weakness, spasms and poor reproduction or denoted by premature births and retained placentas. The blindness is due to atrophy of the optic nerve where it passes through the optic foramen apparently due to pressure atrophy caused by improper development of the foramen. Corn silage, timothy hay, and cod liver oil contain the factor or factors necessary to prevent this type of blindness. The evidence indicates that rations low in calcium and vitamin D are not directly responsible for this type of blindness. Six calves fed 10,000 units of vitamin A in the form of "caritol" developed blindness.

*P11. A study of the malnutrition incident to the heavy feeding of cottonseed meal.* S. I. BECHEL AND S. R. SKAGGS, Pennsylvania State College.

The object of this research was to discover whether or not there is a definite relation between cottonseed meal injury and vitamin A in the diet, as suggested by several workers during recent years.

Twelve Holstein heifers ranging in age from 6 to 18 months were fed on a ration practically devoid of vitamin A and high in cottonseed meal.

The ration consisted of 37.5 per cent cottonseed meal, 37.5 per cent dried beet pulp, 12.4 per cent hominy feed, 12.4 per cent ground barley, 0.7 per cent salt and 0.1 per cent irradiated yeast. No hay was included in the ration.

As soon as definite symptoms of characteristic cottonseed meal injury appeared, each animal was started on a course of treatment designed to effect improvement and cure.

A preparation of pure crystalline carotene dissolved in cottonseed oil was used in the treatment of most of the animals. The dosage of carotene reckoned as A. D. M. A. Vitamin units ranged from about 15,000 per day up to about 120,000 per day. The effect noted did not increase in proportion to the dose fed although some increase was shown.

Some of the animals received activated ergosterol in addition to the carotene and one animal was treated for 30 minutes daily with a carbon arc lamp. Vitamin D did not seem to be a significant factor, judging from growth curves.

Cod liver oil was fed to three of the animals and devitalized cod liver oil was fed to two others.

Alfalfa hay and a poor quality timothy hay were fed to some of the animals late in the experiment.

One animal which received no curative treatment died in 213 days.

Three animals went blind while being treated with the carotene concentrate. This preparation seemed to furnish the necessary factor for growth but did not give adequate protection against the nervous symptoms accompanying cottonseed meal injury.

One animal was completely cured by feeding five pounds daily of a very poor quality timothy hay. Dehydrated alfalfa hay of high quality brought about rapid recovery when as little as three pounds per day were fed.

Cod liver oil brought about rapid recovery from all symptoms of injury. Devitalized cod liver oil seemed to have no effect.

A colorimetric study of the blood carotene showed a graduate decline of carotene in the blood of the animals on the experimental ration, even though receiving large doses of carotene concentrate daily.

The results of this study indicate that vitamin A is probably important in preventing cottonseed meal injury, but that there may be other unknown factors entering in.

Results also indicate that under certain conditions carotene is probably not utilized in the animal body. Further experiments are being conducted along this line.

- P12. Influence of the ration on the vitamin C content of milk.* W. H. RIDDELL, C. H. WHITNAH, AND J. S. HUGHES, Kansas State College.

Titration tests were made on milk from cows fed widely different rations. One group of cows was receiving a ration of Russian thistle hay, molasses, and a grain mixture—a second group, alfalfa hay and a grain mixture—and a third group, alfalfa hay, corn silage, and a grain mixture. After a series of tests had been run on milk from these cows, part of the third group were turned on good rye pasture, and further tests made.

The milk of each cow was tested for ascorbic acid by clarifying with trichloroacetic acid and titrating the serum with 2-6-dichlorophenolindophenol. Average values were calculated for each group.

No significant difference was found in the ascorbic acid content of the milk from the four groups. These results confirm previous indications from biological tests.

- P13. Effect of the condition of corn plant at cutting upon the carotene content of silage.* E. A. KANE AND C. A. CARY, Bureau of Dairy Industry, United States Department of Agriculture.

A number of determinations of the carotene in the corn silage in two of the silos at Beltsville were made in the spring and summer of 1934. The results showed a considerable variation. In order to determine some of the factors influencing the carotene content of corn silage, we, in the fall of 1934, noted the condition of the corn plant at the time of cutting and took samples of the material as it entered the silos from the silage cutter. One portion of each of these samples was analyzed immediately for carotene; and another was put into a coarse-meshed bag, labeled, placed in the silo, and analyzed later when it was uncovered as the silage was used.

The experiment is not complete, but apparently the results show clearly that the carotene content of the corn plant and of the silage made from it depends upon the greenness of the plant at cutting. The silage from the corn that was 90 to 100 per cent green at cutting contained 111 to 156 (average 128) parts of carotene per 1,000,000 of silage (dry weight). When the plant was older and drier and only 40 per cent green at cutting, the carotene content of the silage was only 35 parts per 1,000,000; and, after a light frost when the corn plant was only 20 per cent green, the carotene content of the silage was negligible (i.e., 4 parts per 1,000,000).

Although a carotene determination may not be a reliable measure of the vitamin A value of the yellow corn kernel, it is quite likely that a carotene determination is the best measure available of the vitamin A potency of the corn plant as a whole and also of the corn silage, as most of the carotene occurs in the leaves. We plan further work bearing upon this point.

*P14. The stack silage method of preserving forage crops and the comparative nutritive value of oat and pea silage made in a stack and in a tower.* J. C. KNOTT, R. E. HODSON, AND R. R. GRAVES, Washington State College and Bureau of Dairy Industry, U. S. D. A.

Oats and peas were cut for silage. A 30-ton wood stave silo was filled with this material and a stack was built of like material. The oats and peas were chopped by a silage cutter in both cases. The stack was covered with dirt to a depth of about 18 inches. Each type of silage had a pleasant acid odor and was palatable to both cows and sheep.

Digestion experiments were conducted with three yearling wethers on each type of silage. The chemical composition of the dry matter was practically the same for both types of silage. The stack silage contained approximately 20 per cent more moisture than the tower silage.

The digestibility of the crude protein in the stack silage was approximately 17 per cent lower than in the tower silage. The digestibility of the crude fiber was approximately 11 per cent lower in stack silage. While the digestibility of both the ether extract and nitrogen-free extract was slightly lower in the stack silage, the difference was too small to be significant.

*P15. Pea vine silage as a feed for dairy cattle.* J. O. TRETSVEN, Montana State College.

In 1930 the Montana Experiment Station started a series of experiments to determine the feeding value of pea vine silage or pea cannery refuse for dairy cattle. Two feeding trials were conducted with producing dairy cows. In both trials the cows were divided into two uniform groups of eight head each. With one lot of cows, pea vine silage was substituted for nearly two-fifths of the alfalfa hay fed the other group, otherwise the two rations used were the same. The concentrate used was a mixture of ground wheat, oats, and barley, wheat bran and cottonseed meal, plus 1 per cent bone meal and 1 per cent salt. To eliminate any difference in the productive capacity of the two groups, these trials were conducted by the reversal method. The silage used was of fairly good quality.

Two feeding trials were conducted with growing dairy heifers in which pea vine silage was compared with alfalfa hay. In these trials, yearling heifers were divided into two uniform groups. One group was fed all the good alfalfa hay the animals would clean up and a limited amount of grain. The other group was fed all the pea vine silage they would consume, a limited amount of alfalfa hay and the same grain ration as the first group. The grain in live weight was almost equal for both lots, averaging a little more than a pound per head daily.

From these four trials we may conclude that a good grade of pea vine

silage has a feeding value for milk production and also in the ration for growing heifers when used as in these trials, about one-third that of good alfalfa hay.

Though pea vine silage has a rather strong, penetrating odor, a good quality of milk may be produced by feeding after milking and removing the milk from the barn as soon after drawing as possible.

*P16. Studies regarding the use of mineral acids for the preservation of forage.* Dairy Department, Ohio Agricultural Experiment Station, Wooster, Ohio.

A second crop of green forage, mostly alfalfa and clover, was cut into two  $8 \times 22$  wooden silos with the addition of a 1:4 mixture of 2 N sulphuric and hydrochloric acids, on August 2 and 3, 1934.

It was planned to add 70 liters (about 154 pounds) of this acid mixture per ton of forage. The acid was siphoned into the blower (not a good practice). The amount of acid added in this manner was about  $\frac{3}{4}$  the desired amount, so that the average pH of the resulting silage was 4.31, instead of 4.0, or slightly less acid than planned.

After settling and leveling, the silage was covered with roofing paper and several inches of wet shavings, the loss from spoilage being similar to corn silage handled in the same way.

A similar lot of forage was made into hay which was chopped and blown into the mow.

Four groups of four cows each were used for the feeding comparisons. All received the same grain mixture according to production. Cows fed the acid silage received four ounces and the check group 2 ounces of  $\text{CaCO}_3$  daily.

One group received the acid silage as the sole roughage continuously while another was fed the dry chopped hay continuously in comparison. The other two groups received corn silage at the rate of two pounds per cwt. and were reversed between acid silage and long alfalfa hay as the remaining roughage in periods of 6 weeks. The acid silage was fed *ad libitum* and the hay feeding regulated to supply the check group with about the same amount of dry matter.

The difference in production between the acid silage and the hay fed groups was very small. Marked changes in the bicarbonate and ammonia content of the urine were evident but there was no significant change in the alkaline reserve of the blood.

The vitamin A and carotene values per unit of fat produced were almost identical for the different groups at the same stage of the experiment. The carotene content of the butter fat declined from 7.5 to 3.5 milligrams per kg. during the first two months and remained nearly constant thereafter.

The milk produced on the acid silage feeding seemed to allow somewhat better growth than did that from the hay feeding, both being mineralized by the addition of iron, copper and manganese, but the difference was not outstanding.

*P17. Molasses as a preserving agent in making soybean silage.* E. C. ELTING, Clemson College, South Carolina.

In the test herein reported blackstrap molasses was employed as a preserving agent in the making of soybean silage.

Immature Mammoth Yellow soybeans, cut in the early bloom stage, were ensiled late in August of 1934. One per cent by weight of blackstrap molasses, diluted with an equal weight of water, was added to the soybeans at the cutter. This immature crop had a dry matter content of only 21.25 per cent.

This silo was opened on November 9, 1934, and after removing the surface layer of about nine inches, the silage was found to be of excellent quality, having a clean acid odor and a dark green color. No evidence of putrefaction could be detected.

A group of 16 milking cows was fed this silage as the only roughage for a period of 38 days, including a 10 day transition period and a 28 day experimental period.

This silage was palatable and very little was refused from the start of the trial. Large quantities of the soybean silage were consumed with practically no refusal at the level fed.

Due to the very high moisture content of the silage a comparatively small percentage of the total digestible nutrients required was secured from the silage and the rate of grain feeding appears unusually high.

The silage was not excessively acid, having a titratable acidity equivalent to 1.22 per cent lactic acid.

Comparative analyses of the fresh soybeans and the soybean silage indicated a loss of about 15 per cent of the crude protein during the ensiling process with slight increases in the per cent of ash and crude fiber.

Positive balances for both calcium and phosphorus were secured in a trial with two heavy milking Holstein cows receiving soybean silage as their only roughage. The grain supplement fed contained one per cent salt but no other mineral ingredients.

The soybean silage is very similar to corn silage in percentage of total digestible nutrients when compared on a dry matter basis.

No objectionable flavors or odors were imparted to the milk of cows receiving soybean silage as their only roughage, even when fed immediately before the milking period.

*P18. Experimental cottonseed meal ration plus silage versus herd ration, hay, and silage feeding.* R. H. LUSH, Louisiana State University.

In earlier work at this station five heifers averaged 11,730 pounds of 4 per cent-fat-corrected milk in ten months on experimental ration consisting of either cottonseed meal or soybean meal 25 parts, yellow corn 75 parts, oyster shell flour 2 parts, and one part of salt with corn and soybean silage or pasture as the only roughage. Their production the following lactation on a herd mixture consisting of 40 parts corn, 20 parts each of oat bran, cottonseed meal, mixed hay, one part of salt, and silage was only 8,996 pounds of milk. During the same periods other heifers, receiving the same herd ration, averaged 9,353 and 8,613 pounds, respectively, of 4 per cent milk. Subtracting the 740 pounds seasonal difference gives a net increase of 15.4 per cent for the experimental ration, silage and no hay, over the herd ration, mixed hay and silage. The net return per cow per lactation over feed cost was also \$18.21 higher without hay.

Since then, three double reversal comparisons of 61 to 80 day periods each have been made using the experimental cottonseed meal ration and silage against one-half as much silage, legume hay, and the above herd ration. Mature fresh Holstein cows and corn and soybean silage were used all three years. Five cows were used in each group for the first two trials, and four each in the third. Grain was fed at the same rate to both groups. Silage was fed at the rate of six pounds alone, or three pounds plus one of hay daily per 100 pounds live weight.

Dehydrated soybean hay was used in the first trial, but was not very palatable. During the silage alone periods 2.36 per cent more 4 per cent milk was obtained. There was also less loss in live weight than when hay was fed. This trial indicated 281 pounds silage was equivalent to 100 pounds soybean hay. In the second trial, western alfalfa hay of good quality was fed, and 8.22 per cent more milk obtained for hay and silage feeding than with silage as the only roughage with less loss in live weight. This trial indicated 386 pounds of silage were necessary to replace 100 pounds alfalfa hay. In the third trial, locally grown dehydrated alfalfa was used, with 1.66 per cent more milk being produced, but greater loss in weight for hay and silage periods than for silage alone. This indicated that 324 pounds of silage were necessary to replace 100 pounds of this hay, or an average of 330 pounds for the three tests to equal 100 pounds legume hay, if no difference existed in the nutritive value of the two grain rations. The average of the three trials was 28.89 pounds milk per cow daily on experimental ration and silage and 29.44 pounds on the herd ration, legume hay, and silage, or an increase of 1.9 per cent for the latter method of feeding. Because of high cost of legume hay, oats, and bran, feed cost of 100 pounds milk averaged 10 cents less on silage alone than with legume hay.

These trials have demonstrated that under our conditions, legume hay and a bulky grain ration are not necessary for economical production when silage, corn, cottonseed meal, and adequate mineral matter are fed.

*P19. The digestibility and feeding value of Russian thistle hay.* H. W. CAVE, W. H. RIDDELL, AND J. S. HUGHES, Kansas State College.

Due to the drought and extreme shortage of feed in the western half of Kansas and portions of several other states, many thousands of tons of Russian thistles have been harvested and fed to livestock during the past season. Little information was available as to the digestibility of Russian thistles and practically nothing was known as to their feeding value. This report contains the results of a digestion trial where thistles were fed as an exclusive diet to dry cows and the results of a feeding trial where thistles supplied the roughage portion of the ration for lactating cows.

In the digestion trial one representative each of the Ayrshire, Holstein and Jersey breeds were used. The length of the trial was 25 days, divided into a 15-day preliminary period and a 10-day collection period. The amount of thistles fed varied from 4,000 grams daily fed to the smallest cow to 7,000 grams fed to the largest cow. These amounts of the hay were sufficient to approximately maintain the body weights of the experimental animals.

The hay used in both the digestion trial and the milk production trial was cut in early August while still fairly green and was baled as soon as possible. This resulted in a small amount of mold in a few of the bales.

The results of the digestion trial are given in the following table.

*Composition, digestibility, and digestible nutrients of Russian thistle hay*

	DRY MATTER	CRUDI PROTEIN	FILIFR FIBRAC I	CRUDE FIBER	ASH	N FREE EXTRACT
Composition, per cent	86.22	9.25	0.96	22.50	15.65	37.86
Apparent digesti- bility, per cent	54.8	63.2	40.3	44.6		61.7
Digestible matter per cwt.	47.25	5.85	0.39	10.04		23.36

In the milk production trial to be reported, two lots of 5 cows each were fed by the double reversal method through three 30-day periods. One group of cows received ground alfalfa hay as a source of roughage, while the other was receiving ground Russian thistle hay. The grain ration used with alfalfa hay consisted of 4 parts corn and 1 part wheat bran. That fed with thistles consisted of 2 parts corn, 1 part bran, and 1 part cottonseed meal. To each of these grain mixtures was added 1 per cent each of steamed bone meal and common salt. To improve the palatability and

increase the energy of the thistle ration, each cow while receiving the thistles was given 4 pounds daily of black strap molasses.

**P20. *Lespedeza Sericea* feeding trials with dairy cows.** C. E. WYLIE AND S. A. HINTON, University of Tennessee.

*Lespedeza Sericea* is a relatively new perennial legume in the United States. It has been grown at the Tennessee Experiment Station since 1927. (Bul. 154.) Its high protein content (11.5–14.5%), high mineral content (4.1–4.6%), together with its high yield and other characteristics make it a desirable plant in considering feeds for dairy cows. Several feeding trials with a limited number of cows were made during the winters of 1933–34 and 1934–35

In the 1933–34 trials it was compared with mixed alfalfa hay fed *ad libitum* with 10 pounds of concentrates and 20 pounds of corn silage. Milk and fat records and weight of cattle were kept as well as weights of feed. In this trial the cows consumed less *Lespedeza Sericea* than mixed alfalfa hay and produced slightly less milk and fat. The most significant fact was that the cows did not seem to relish or eat as much *Sericea* hay as was expected. The trials the following year were planned to find a solution to this difficulty. The following 20-day trials with two Holsteins and one Jersey were made with fixed amounts of silage (30 lbs.) and concentrates (10 lbs.):

Trial I. *Lespedeza Sericea*—*ad libitum*.

II. Alternate days of alfalfa and *Sericea ad libitum*.

III. Alfalfa and *Sericea* ground equal parts—fed *ad libitum*.

IV. *Sericea* ground and mixed with molasses (10 lbs. to 1 lb.).

*Results—20 day periods*

TRIAL	HAY CONSUMED	MILK PRODUCED	FAT PRODUCED	GAIN IN WEIGHT
	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>
I	<i>Sericea</i> 268.3	2204.4	81.72	63
II	<i>Sericea</i> : alfalfa 88.5 : 249.9	1946.4	78.32	39
III	681.0—Equal parts <i>Sericea</i> and alfalfa	1774.1	72.77	86 -
IV	<i>Sericea</i> and Molasses 360.4	1392.3	62.30	77 -

**P21. The effect of soybeans on the fat content of milk.** J. W. WILBUR, J. H. HILTON, AND CO-WORKERS, Purdue University.

The question of the effect of feeds on the fat content of milk is especially interesting from the standpoint of feeding soybeans. In a series of seven feeding trials in which rations containing soybeans in varying amounts

were compared with rations which did not contain soybeans, the fat test of the milk was increased approximately 0.25 per cent. A total of 26 cows were fed on each ration during these feeding trials.

An index figure to show the relationship between milk and fat was obtained by dividing figures representing the percentage of original fat production by figures representing the percentage of original milk production, the original milk and fat being the amount of production during the first 10 days of each feeding trial. The percentage of original production was obtained by dividing productions during the first or original 10-day period into the productions during the subsequent 10-day periods. When no soybeans were included in the ration the index figures averaged 96.4, indicating that the amount of fat in the milk was low in relation to milk production. When soybeans were included in the ration the average index figure was 107.7 indicating that the fat production was high. These two index figures show that soybeans actually increased the percentage of fat either by maintaining the fat content of milk while milk production was declining or by actually increasing the fat content of milk either when milk production increased or declined.

*P22. The effect of quality and level of protein intake upon growth and milk production.* I. W. RUPEL, G. BOHSTEDT, AND E. B. HART.  
University of Wisconsin.

To study further the economy as well as the limitations of "home grown" dairy rations this experiment was planned and started in January, 1934. Six lots of heifers, three animals per lot, were started at six months of age on the following rations:

- I—*Inadequate Protein.* Timothy hay, corn silage; corn 50, and oats 50.
- II—*Adequate Protein.* Timothy hay, corn silage, corn 37, oats 37, and corn gluten meal 26.
- III—*"Mixed Protein."* Timothy hay, corn silage; corn 266, oats 266, wheat bran 234, linseed meal 117, and gluten meal 117.
- IV—*"Home Grown."* Alfalfa hay, corn silage; corn 50 and oats 50.
- V—*"Home Grown," plus a phosphorus-rich feed.* Alfalfa, corn silage; corn 60, oats 20, wheat bran 20.
- VI—*"Home Grown," plus mineral phosphorus.* Alfalfa, corn silage; corn 500, oats 500, bone meal 18.5.

It is planned to carry the animals through the growing period and through at least two lactations on their respective rations. Until first calving the ratio of the feeds will be as 1.0 for hay, 1.0 for concentrates and 2.0 for silage. The nutritive ratios during growth will be 1:10.7 for Lot I, and 1: 6.7 for the other lots.

When the animals come into milk it is planned to adjust the proportion of the concentrates so that a constant ratio of protein and total digestible nutrients in the ration to the milk yield, will be fed above maintenance to all animals except those in Lot I.

The first year's results show the following rates of average daily gain respectively for the six lots: 1.28, 1.36, 1.33, 1.48, 1.46, 1.52. While other differences appear insignificant all alfalfa-fed lots averaged to gain faster than the timothy hay lots. Analyses on the blood calcium and phosphorus made after 9 months on experiment showed normal concentrations of these elements for all animals.

*P23. Milk and butterfat production on high and low protein rations.*

C. A. CARY, Bureau of Dairy Industry, United States Department of Agriculture.

Two mature Jersey cows have been fed during different lactation and preceding dry periods on a ration that was low in protein content (*i.e.*, it contained 0.5 lb. of crude digestible protein per 1000 lbs. live weight of cow for maintenance and 1.25 times as much protein as occurred in the milk to cover the requirements for milk secretion). During other lactation and dry periods they were fed a ration of exactly the same composition except that the protein was increased by substituting a good grade of commercial casein for carbohydrate. The casein that was thus introduced into the basal ration was 0.2 lb. per 1000 lbs. of live weight, plus an amount equal to 0.75 of the protein in the milk. The energy consumption in both cases was about 10 per cent above the Savage Standard.

When changed from the high- to the low-protein ration at the beginning of her dry period, one cow immediately in the succeeding lactation produced 50 per cent. less milk and fat. The other cow maintained her production for nearly 8 months during the succeeding lactation, at the end of which time the milk yield and percentage of fat in it dropped abruptly. In her next lactation on the low-protein ration, her milk yield was 22 per cent of that on the preceding high-protein ration and the fat production was less than 15 per cent. She went dry in 5 months.

When these changes of ration were reversed both cows immediately in their succeeding lactation periods fully resumed their original higher level of milk and fat production. The average body weight of one cow was about 100 pounds lower on the low-protein feeding. With the other cow the changes in body weight were negligible.

*P24. Extremes in protein feeding—Bearing of the results on the protein-feeding standard.* A. E. PERKINS, Ohio Agricultural Experiment Station, Wooster.

Rations of N. R. 1:2 and N. R. 1:13, and others less extreme, have been used over extended periods.

In the 1:2 ration we seem definitely to have passed the point where additional protein is of any advantage. The amount of protein supplied above maintenance by this ration was approximately six times the protein

content of the milk. The amount of production was less than would normally be expected from these cows on ordinary feeding, but there was no definite evidence that the ration was injurious to the cow.

On the rations of opposite type N. R. 1:13 there must have occurred over long periods practically 100 per cent conversion of feed protein to milk protein, even assuming a maintenance requirement of 0.55 pound instead of the conventional 0.70 pound per 1000 pounds live weight.

Production on this type of feeding was on a lower plane and the cows were much thinner in flesh than normal but maintained their live weight from year to year, remained in apparent health, and produced normal well-developed calves. Clearly, the dairy cow has a remarkably wide degree of tolerance with respect to the protein content of her ration.

Cows in these experiments on rations having a N. R. of 1:11 for two successive lactations also apparently made 100 per cent transformation of feed protein to milk protein, after deducting the conventional maintenance allowance. Satisfactory production and condition were apparently maintained.

The old question which has long troubled the makers of feeding standards as to how completely feed protein could be transformed into milk protein has apparently been answered, at least for rations containing a reasonable variety of feeds, such as present in these rations.

The relation of these results to the present feeding standard situation will be briefly discussed.

*P25. Changes in weight of new-born calves as related to the method of feeding.* C. L. COLE, University of Minnesota.

Fifty-three Guernsey calves were used and divided into three groups. They were all weighed at birth and at 24-hour intervals thereafter until they reached the age of 14 days.

Group I consisted of thirteen calves that were taken from their dams immediately following birth without having nursed. They were hand fed with their dam's milk at all times.

The twenty-six calves of Group II were left with their dams 48 hours after birth. Food was then withheld for 24 hours and they were hand fed thereafter.

The third group contained 14 calves that were left with their dams 96 hours and then cared for in the same manner as Group II.

Group I suffered a slight weight loss for the first two days and then a continued gain for the balance of the fourteen days at which time they showed an advantage of 7.9 pounds and 10.5 pounds in average weight increase over groups II and III respectively.

Group II made a daily gain for the first two days, then a decided loss on the third day and a daily increase for the balance of the 14 days.

Group III made a daily gain for the first four days. The next three days showed a daily weight loss and then a daily gain for the balance of the 14-day period.

Group II made a slightly larger average gain during the course of the experiment than did Group III.

The experiment clearly shows that dairy calves do not suffer a natural weight loss following birth. Also that calves not allowed to nurse their dams seem to get a much better start during their first two weeks than those allowed to nurse.

They are also much easier to teach to drink when never allowed to nurse.

*P26. The nutrients required by dairy cows kept in an open shed vs. cows kept in a dairy barn. J. R. DICE, North Dakota Agricultural College.*

Animal husbandmen have been prone to assume that farm animals should be comfortably housed for maximum production and for economical production. It is common knowledge now that beef cattle kept in open sheds will make more economical gains than barn fed cattle but the idea prevails that "thin skinned milk cows" require rather warm quarters.

Our first progress report showed that for three, two-month periods in different years, the cows kept in open sheds were somewhat more persistent producers than similar groups housed in the dairy barn and that milk cows can stand low temperatures provided they have access to shelter that is dry and free from drafts.

This progress report gives results obtained by comparing two groups of cows kept in the dairy barn to similar groups housed in a pen barn. The average temperature of the barn for two trials was 48.2° F., for the shed 28.3° F. The average humidity of the barn for the last period was 84.3, for the shed 75.9.

During the two five-months periods beginning November first, the eleven cows kept in the barn produced 38,706.5 pounds of 3.88 per cent milk and 1,513.5 pounds of butterfat. The shed cows produced 35,824 pounds of 4.53 per cent milk and 1,624.05 pounds of butterfat.

The barn group consumed 2.499 pounds of digestible protein for every pound of butterfat produced and 0.0977 pound of protein for each pound of milk. The shed group required 2.18 pounds of protein for a pound of butterfat and 0.0986 pound for a pound of milk.

The barn cows used 16.03 pounds of total digestible nutrients for a pound of butterfat, and 0.629 pound for a pound of milk. The shed cows consumed 14.02 pounds of T. D. N. for a pound of fat and 0.635 pound for a pound of milk. The difference in the test of the milk may have had some influence on the nutrients required for fat production.

The barn groups weighed 13,400 pounds at the beginning of the trial

and gained 751 pounds in five months. The shed groups weighed 12,352 pounds and gained 982 pounds.

During the 1934-35 trial period three cows in the barn and three in the shed produced approximately the same amount of milk and butterfat. The three barn cows produced 13,206 pounds of 3.695 per cent milk and 488 pounds of fat. They used 0.0878 pound of protein and 0.673 pound of T. D. N. for every pound of milk and 2.397 pound of protein and 18.38 pounds of T. D. N. for every pound of fat produced. They gained a total of 271 pounds.

The three shed cows produced 12,145 pounds of 3.98 per cent milk and 483 pounds of fat. They consumed 0.0897 pounds of protein and 0.689 pounds of T. D. N. for every pound of milk and 2.253 pounds of protein and 17.31 pounds of T. D. N. for every pound of fat produced. They gained 260 pounds.

These data do not seem to indicate that the cows in the shed used nutrients fed them to maintain body temperatures.

*P27. Formulae for calculating rations for milk cows.* A. H. KUHLMAN, Oklahoma A. & M. College.

The following formulae based on the Morrison Feeding Standard eliminate the necessity of constant reference to the feeding standard, and shorten the work involved in calculating rations for milk cows:

$$X = M(.0311 + .0072F) + 0.7 \frac{W}{1000} \quad (1)$$

$$Y = M(.1005 + .057F) + 7.925 \frac{W}{1000} \quad (2)$$

In these equations  $X$  = pounds of digestible crude protein required daily,  $Y$  = pounds of total digestible nutrients required daily,  $M$  = pounds of milk produced daily,  $F$  = per cent fat in milk, and  $W$  = body weight of the cow.

While the values for body maintenance are identical with those of the Morrison Feeding Standard, the nutrient requirements for milk production approximate very closely the averages of the upper and lower limits for any given fat test specified in the Morrison Standard.

If a concentrate mixture is used which adequately meets the protein requirements, when roughages are fed in proportion to body weight, the pounds of concentrates ( $Z$ ) required daily are expressed by the following formula, derived from the second equation:

$$Z = M \left( \frac{.1005}{A} + \frac{.057F}{A} \right) - \left( \frac{B - 7.925}{A} \right) \frac{W}{1000} \quad (3)$$

In this equation  $A$  = pounds of total digestible nutrients in one pound

of concentrate mixture and  $B$  = pounds of total digestible nutrients fed daily in the roughage allowance per 1000 pounds body weight.

The specific formula for any given roughage and concentrate mixture may be derived readily from equation (3). If alfalfa hay and corn silage are fed at the rate of one and three pounds respectively per 100 pounds of body weight and if the concentrate mixture contains 73 per cent T. D. N., the formula is

$$Z = M (.1377 + .078F) - .00349W \quad (4)$$

Equations (1) and (2) provide very useful short methods for determining the daily requirements of nutrients for milk cows, while specific formulae derived from equation (3) are especially valuable in saving time in calculating the daily allowances of a concentrate mixture in feeding experiments. A further advantage is that all calculations involved in equations (1), (2), and (4) may be made with calculating machines.

*P28. Permanent records in the station herd.* R. B. BECKER, University of Florida.

What forms of permanent records shall be kept in a station dairy herd, in order to have them in concise convenient form for research purposes?

The above question was decided by the late Prof. A. C. Baer and his associates at the Oklahoma station a few years ago. Two forms were decided upon, drafted, and printed, and the station records kept thereon. The first of these forms permitted daily milk yields of individual cows for each lactation, to be kept in such manner that these could be tabulated and analyzed either by calendar months or by lactation curves, using any arbitrary unit from the date of calving.

The second form is a loose-leaf pocket herdbook in which each cow's record is tabulated by calendar months and lactation totals for four lactations. Space is provided for registered name and number, date of birth and a two-generation pedigree; also for color markings, tattoo and herd number. Official records for four lactations may be listed. Breeding and reproduction records for four breeding seasons are included. Since the average life-span of most dairy cows does not exceed four lactations, this form is adequate. For cows that exceed this age, as many as four additional record sheets have been used. The date and cause of disposal are the last entries made on each record.

Since these are loose-leaf forms, the records are filed permanently in the dead file as animals leave the herd. This sheet is of convenient size for sorting and tabulating the breeding, gestation and calving records.

Other stations have designed record systems adapted to their special uses. It is felt, after ten years' use of these record forms, that they have proved their usefulness as an integral part of a permanent record system.

*P29. Effect of breeding efficiency and culling on herd production.* LYNN  
COPELAND, American Jersey Cattle Club, New York City, N. Y.

The percentage of dry cows in Jersey herds on Herd Test has been found to be directly proportional to the herd average. The percentage of dry cows carried in all herds averaged 15.39. This means that on an average each cow in the herds was dry 56 days during each year. Herds averaging less than 250 pounds of butterfat showed an average percentage of dry cows of 28.23, while in the herds averaging over 500 pounds of butterfat, the percentage of dry cows each year was only 10.72. Small and medium size herds contain a smaller percentage of dry cows than do the larger herds.

Herds of widely different levels of production show very similar frequency curves in the distribution of cows on the basis of production. Culling 10 per cent of the poorer producers results in an increase in herd average of approximately 20 pounds of butterfat and culling 25 per cent of the low producing animals increased the herd averages approximately 42 pounds of butterfat. The increases in average yield resulting from culling was nearly constant regardless of the initial average production of the herds.

## MANUFACTURING SECTION

- M1. Studies on the ash and nitrogen distribution of processed cheese as affected by the salts used and a comparison of the methods used for the determination of the pH of the cheese.* HUGH L. TEMPLETON, University of Wisconsin.

This paper presents the results of recent studies on the ash and nitrogen distribution of processed cheese as affected by the kind and amount of the different salts used, as the so-called emulsifiers, in the manufacture of processed cheese. The salts include sodium and potassium citrate, Rochelle Salt (sodium-potassium tartrate) and the sodium salts of ortho, meta and pyro phosphoric acids. In studying the nitrogen distribution only the water and 5 per cent salt soluble fractions have been determined and the values expressed in percentage of the total nitrogen found in the cheese. It has been found that with certain of these salts that there is a considerable portion of the nitrogen which cannot be accounted for in either the water or 5 per cent salt soluble fractions while with other salts nearly all the nitrogen present can be found in these two divisions. In general the percentage of water soluble nitrogen increases with the increase in the amount of the emulsifier used. With the exception of slight increases with the smaller amounts of salts, the 5 per cent salt soluble fraction must of course decrease as the water soluble portion increases. The total ash increases with the increasing amounts of the emulsifiers used. The total ash was divided into the water soluble and insoluble fractions.

In regard to the pH determinations, comparisons are given showing the variations in the values when the determinations are made with the hydrogen electrode on (1:10) aqueous cheese suspensions, and when made with the quinhydrone electrode on the aqueous cheese suspension and when made on a cheese and quinhydrone paste. The results by the first two methods are quite comparable and average about 0.50 of a pH unit more alkaline than the latter method.

- M2. Effects of some ions on the properties of ice cream mixes.* J. I. KEITH, C. W. RINK, AND EARL WEAVER, Oklahoma A. & M. College.

Ice cream mixes of different compositions, which had been carefully standardized, were used in this series of experiments. Each mix was divided into four batches after standardizing. One batch was always used as a control. To each of the other batches was added a salt solution which furnished one of the ions being studied. Water was added in like amount to the control batch. Citrate, carbonate, and calcium ions were studied. The salts furnishing the different ions were used in such amounts that the concentrations of the active ions were the same in all cases. The mixes

were all processed in the same manner except that the ions were added at different stages of processing: (1) before pasteurizing; (2) just before homogenizing; (3) after homogenizing but before cooling; and (4) after aging and immediately before freezing. Results given in this paper are those obtained by studying 24 batches of each kind of mix.

It was found that the citrate ion greatly decreased the viscosity and clumping of fat globules, but decreased the titratable acidity only very slightly. The stability of proteins was increased particularly toward precipitation by alcohol.

The carbonate ion effected the mixes in the same manner as the citrate ion except that it was more active in lowering the titratable acidity and increasing the stability of the proteins toward precipitation by acid.

The calcium ion give exactly opposite results from those of the citrate ion in all particulars. This included an increase in viscosity, fat clumping and acidity and a marked decrease in the stability of the proteins toward precipitation by either alcohol or acid.

*M3. The hydrogen-ion concentration and titratable acidity of butter, cream and buttermilk.* O. F. HUNZIKER AND W. A. CORDES, Blue Valley Creamery Co., Chicago, Ill.

The primary aim of this investigation was to study the influence of commercial practices of neutralization and pasteurization of cream on the hydrogen-ion concentration of butter. Further, it was attempted to ascertain whether or not the pH determination could be used as a dependable index for the prediction of keeping quality in cold storage butter. Keeping quality here refers to freedom from flavor deterioration caused by progressive chemical changes that lead to the development of fishy flavor, storage flavor and allied flavor defects characteristic of butter held in cold storage.

Experience in the commercial manufacture of butter for storage purposes has demonstrated conclusively the dependability of the churning acidity of the cream and cream serum as an index to keeping quality as regards freedom from fishiness. Observations on the development of the less intense but related "storage" flavor indicate the importance of such factors as the quality of the original cream and its titratable acidity upon arrival at the butter manufacturing plant. In endeavoring to apply the pH value of butter to the problem of predicting keeping quality it became necessary to study the relationship between the titratable acidity of both sweet and neutralized cream of different richnesses, and the pH of butter. The titratable acidity of butter, butter serum and buttermilk, and the pH of the cream and buttermilk were also determined.

This investigation consisted of five separate series of trials involving a total of 59 separate churnings. In each series enough fresh sweet milk was separated to provide cream for 11 to 12 churnings, which received the following respective treatments:

Sweet cream, raw, churning acidity .15%  
Sweet cream, pasteurized, churning acidity .15%  
Sweet cream, ripened to a churning acidity of .25%  
Sweet cream, ripened to a churning acidity of .50%  
Sweet cream, ripened to a churning acidity of .80%  
Sweet cream, ripened to .50% acid, neutralized to a churning acidity of .15%  
Sweet cream, ripened to .80% acid, neutralized to a churning acidity of .15%  
Sweet cream, ripened to .50% acid, neutralized to a churning acidity of .25%  
Sweet cream, ripened to .80% acid, neutralized to a churning acidity of .25%  
Sweet cream, ripened to .50% acid, neutralized to .15% and ripened back to a churning acidity of .25%

In the case of the neutralized churnings different portions of the cream ripened to .50% acid were neutralized with calcium-magnesium lime, and with carbonate of soda, respectively. Different portions of the churnings ripened to .80% acid were neutralized with calcium-magnesium lime, and with both calcium-magnesium lime and carbonate of soda, respectively.

Three different richnesses of cream were used, *i.e.*, cream testing 22.5%, 32.5% and 44.5% fat, respectively. The more important results of this investigation are enumerated below.

1. Sweet cream, unpasteurized, yielded butter with a higher titratable acidity and butter serum acidity and a lower pH than the same cream pasteurized. For raw cream these figures averaged: 0.044%, 0.121% and pH 6.23, respectively. For pasteurized cream, these figures averaged: 0.029%, 0.098% and pH 6.34, respectively.

2. Cream with the lowest fat content (22.5%) always yielded butter with a higher pH than the richest cream (44.5%). The cream of medium richness yielded butter with an average pH intermediate between that of thin and rich cream, but there were individual exceptions, where the pH of the butter from the intermediate cream exceeded the limits of the pH from the thin and rich cream.

3. The cream acidity was, as a rule, lower than the buttermilk acidity. The higher the churning acidity of the cream the greater the difference. The difference was greater in the case of unneutralized cream than with neutralized cream, and ranged from an average of 0.005% for neutralized cream churned at 0.15% to an average of .194% for unneutralized cream churned at .792% acid.

4. The cream serum acidity was consistently higher than the buttermilk acidity. The higher the cream serum acidity of unneutralized cream the greater the difference. In the case of unneutralized cream the difference averaged from .025% for cream churned at a serum acidity of .220%, to .242% for cream churned at a serum acidity of 1.228%. The difference

between cream serum acidity and buttermilk acidity was greater in the case of neutralized cream than with unneutralized cream.

5. In the case of sweet, unneutralized cream the pH of both the cream and the buttermilk was higher than the pH of the butter. In the case of all sour, unneutralized cream (above .25% acid) and most churnings of neutralized cream, the pH of the butter was higher than the pH of both the cream and the buttermilk. In all cases the pH of the cream was higher than the pH of the buttermilk.

6. The titratable acidity of butter made from cream with a churning acidity of .25% or less did not exceed .065% with two exceptions, and in most cases stayed below .06%. In the case of cream with a higher churning acidity the titratable acidity of the butter was correspondingly higher.

7. Neutralized sour cream produced butter with a decidedly higher pH than the same cream unneutralized and churned at the same acidity. The wider the range of acid neutralized, *i.e.*, the higher the acidity before neutralization, or the lower the acidity churned at, or both, the greater the difference in pH between butter made from neutralized sour cream and from unneutralized cream of the same churning acidity.

Thus in the case of a churning acidity of .25%, unneutralized sweet cream yielded butter with a pH range of 5.70 to 5.89, averaging 5.79, while portions of the same cream ripened to .80% acid and neutralized back to .25%, yielded butter with a pH range of 6.31 to 6.71, averaging 6.48.

In the case of a churning acidity of .15%, unneutralized sweet cream yielded butter with a pH range of 6.04 to 6.49, averaging 6.29, while the pH of butter made from portions of the same cream ripened to .80% acid and neutralized back to .15%, ranged from 7.0 to 7.26, averaging 7.09.

8. Neutralization from .5% acid to .15% with lime gave a higher pH in the butter than neutralization with soda, *i.e.*, 6.89 as compared with 6.58.

9. These findings definitely show that the character and treatment of the cream have a marked influence on the pH value of the resulting butter. They indicate that portions of the same cream treated differently but churned at the same titratable acidity, may yield butter with widely varying pH values. Therefore, the determination of the pH value would not give any definite indication of the churning acidity or serum acidity of the cream at churning, which is the only dependable means of controlling keeping quality.

10. Cream neutralized with lime or lime and soda and churned at a titratable acidity of .25% gives commercial butter of dependable keeping quality. Such butter may have a pH as low as 6.00, as shown in this investigation. However, when butter is encountered with a pH slightly lower, *e.g.*, 5.70, this may mean poorer keeping quality only if churned from neutralized cream and not if the butter is from sweet cream allowed to sour only to .25% acidity. The highest pH found in these trials in the

case of sweet cream butter was 6.49. Figures obtained in the range 6.5 to 7.0 or slightly above might be expected to indicate butter made from neutralized cream; as a general rule the higher the pH the higher the acidity of the cream before neutralization, and the greater the amount of neutralizer added. Butter of this type may be depended upon to withstand prolonged cold storage without danger of developing "fishy flavor," but it usually shows a more or less marked "storage flavor."

*M4. Range of hydrogen-ion concentration in sour cream butter.* E. H. PARFITT, Purdue University.

Approximately 65 manufacturers of butter in Indiana and neighboring states have been submitting to Purdue University for analysis monthly samples of butter. Upon these samples the hydrogen-ion concentration, expressed as pH, was determined on the serum of the butter, using a quinhydrone electrode.

The range in pH of 468 individual samples was from 8.0 to 4.6 and of all samples 10.03 per cent were over pH 7.0; 25.42 per cent from pH 7.0 to 6.51; 38.23 per cent from pH 6.5 to 6.01; 18.58 per cent from pH 6.0 to 5.51; 4.06 per cent from pH 5.5 to 5.01 and 3.63 per cent below pH 5.0. There was in January, February and March a decided decrease in the number of samples of butter having a serum pH of over 7.0. Some individual plants were able to control the pH of the butter manufactured within narrow limits while others varied greatly.

*M5. The thiocyanogen value as a means of measuring unsaturated fatty acids in butterfat.* O. J. HILL AND L. S. PALMER, University of Minnesota.

A study has been made of the thiocyanogen value in measuring the unsaturated fatty acids of butterfat. The thiocyanogen value makes possible the determination of fatty acids less saturated than oleic acid. It was found necessary to modify the method of Kaufmann,<sup>1</sup> Zeleny and Bailey,<sup>2</sup> somewhat in order to secure satisfactory results on butterfat.

Some workers have calculated the values in terms of linoleic acid. This calculated value cannot accurately determine the linoleic and linolinic acids from a mixture containing both of these acids. The difference between the iodine value and the thiocyanogen value were used in this study to express the amount of fatty acid less saturated than oleic acid.

This determination was applied to a series of butter samples. These samples were obtained from cows fed a basal ration low in fat. Oils were

<sup>1</sup> Kaufmann, H. P. Die Rhodanometrie von Fetten und Fettgemischen. Ztschr. Untersuch. Nahr. u. Genussmtl. 51: 15-27.

<sup>2</sup> Zeleny, L., and Bailey, C. H. Thiocyanogen number and its application to studies on lard. Indus. and Engin. Chem. 24: 109.

added to the basal ration in order to observe changes resulting in composition of the butterfat. The values in butterfat produced from the basal ration when oils were not fed ranged from 2.49-4.16, whereas the basal ration supplemented with linseed oil gave values from 4.37-5.79. Corn oil or cottonseed oil feeding failed to increase the values above the range for the basal ration. Coconut fat decreased the value.

It appears that the thiocyanogen value may be useful in studying the effect of feeds on the composition of butterfat.

*M6. The effect of homogenization on some of the fat constants of milk.*

I. A. GOULD AND G. M. TROUT, Michigan State College.

In order to ascertain the chemical changes which may occur in the fat of milk homogenized prior to and after pasteurization, purified butterfat was secured from pasteurized and raw milk which was homogenized at 1500 pounds pressure. The butterfat was examined for changes in the Reichert-Meissl and Polenske Numbers, the refractive index, and the acid degree.

Averages of five trials showed homogenization of either raw or pasteurized milk had no appreciable effect on the Reichert-Meissl or Polenske Numbers, or on the refractive index of the fat. However, when raw milk was homogenized, the acid degree of the fat (expressed as cc. of 1 N.NaOH necessary to titrate the free acids in 100 grams of fat), was increased from .572 to 2.231 within a few minutes and to 10.37 within twenty-four hours. With an increase in the acid degree of the fat the titratable acidity of the raw milk increased and the pH decreased. Pasteurized homogenized milk showed none of these changes.

In three trials, the acid degree of the fat from raw homogenized milk was determined at the end of each twenty-four hour period for 120 hours. The greatest daily increase occurred during the first twenty-four hours, when the acid degree increased on the average from .566 to 9.92. The increase in free acids was continuing at the end of 120 hours, even though the acid degree averaged 16.35, or an equivalent of 1.4388 grams of butyric acid, at that time.

*M7. Variations in physical properties of milks.* G. P. SANDERS, K. J.

MATHESON, AND L. A. BURKEY, Bureau of Dairy Industry, United States Department of Agriculture.

The following tests were made on the fresh milk of each cow in a herd of 150 animals: pH, percentage of casein, Hill curd test, rennet coagulation time, rennet curd tension measured by means of the American Curd-O-Meter (Hill curd tester), firmness of rennet curd, alcohol test, and certain tests for evidences of mastitis. The Hill curd test was found not satisfactory for measuring the firmness of curd for making Swiss cheese. The test for rennet coagulation time was found to be much more satisfactory for this purpose; the rennet curd tension test was found to be even more suitable.

The pH of the milks influenced the Hill curd test results slightly and bore a much more definite relation to the rennet coagulation time, the rennet curd test, the alcohol test, and the tests for mastitis.

The average rennet curd firmness was definitely increased by an increase in the percentage of casein. Milks stable to rennet were usually stable to alcohol. Stability to both rennet and alcohol was found to be increased by storage at 2° to 5° C. As in the case of the rennet tests, added  $\text{CaCl}_2$  caused a loss of stability in the alcohol test.

In milks showing evidences of mastitis the rennet curd was usually but not always soft; some soft-curd milks showed no evidence of mastitis. A decrease in percentage of casein as a result of mastitis was not generally found.

*M8. Soft curd character induced in milk by intense sonic vibration.* LESLIE A. CHAMBERS, University of Pennsylvania.

Reduction in the curd tension (Hill test) of milk was accomplished by flowing the fluid in a thin layer over electromagnetically driven diaphragm sources of intense sonic vibration.

A study was made of the curd reducing effectiveness of oscillators operating at 1100, 1200, 2160, 3000, 610, and 360 cycles as related to original curd tension of the milk, temperature, time of exposure (velocity of flow), acoustic energy output, and variations in the mechanical features of the apparatus.

The percentage reduction in curd tension was greatest in hard curd (60 grams and more) milks. Final curd values approached a constant level in the soft curd range no matter what the original curd texture.

No reduction in curd tension was obtained in milk treated below 18° C. and very little at temperatures below the melting point of butterfat.

It was found that one of the oscillators (360 cycle) was most efficient when the milk flow was maintained at 250 gallons per hour. With a sound output of about 900 watts more than 50 per cent reduction was attained.

With the 360 cycle oscillator the degree of reduction in curd tension was found to bear a direct linear relationship to the power input up to 2000 watts when the velocity was 250 gallons per hour. This represents an output of about 18 watts per one per cent decrease in tension. Other oscillators at different frequencies showed much the same effectiveness with equivalent outputs per unit volume of milk. Hence differences in frequency were found to be negligible within the range explored.

A direct relationship was found to exist between degree of fat dispersion and degree of curd tension reduction. The increased number of fat particles was shown to influence curd texture by weakening the curd matrix and by providing increased adsorptive area on which protein was fixed.

Since but a small proportion of the total fat in milk need be finely subdivided to reduce the curd tension, a method was devised for producing soft curd milk by vibration without impairing the final cream volume.

*M9. Variations in the curd tension of the milk throughout the complete lactation period.* M. H. BERRY, University of Maryland.

In recent years considerable interest has been manifested in soft-curd milk and its production. Experiment Stations and research laboratories have been investigating several different phases of the curd tension of cow's milk, both as it occurs naturally, and as it is affected by various processing treatments.

With the development of processes whereby soft-curd milk may be produced from milk with a high curd tension, the question of changes in the curd tension of the original milk throughout the year or lactation period become very important.

The work presented in this paper covers a series of tests made to determine the variations in the curd tension of the milk throughout the lactation period.

These tests involved:

1. The curd tension of colostrum.
2. Variations in curd tension between milkings of the same day and between consecutive days.
3. The curd tension of the milk throughout the entire lactation period.
4. The effect of an abortion on the curd tension of the milk.
5. Seasonal variations in the curd tension of the milk.
6. The average curd tension of the milk produced in one lactation period as compared with that of a subsequent lactation period.

The results of the tests indicate:

1. That colostrum has a high curd tension.
2. That there is little variation in the curd tension of the milk between milkings of the same day or between that of consecutive days.
3. That the curd tension of the milk is fairly uniform throughout the lactation period with the exception of that produced the first few days following freshening when the curd tension may be very high.
4. That an abortion during the lactation period does not appear to have any effect upon the curd tension of the milk.
5. That there is not a seasonal variation in the curd tension of the milk.
6. That the average curd tension of the milk produced in one lactation period may vary widely from the curd tension of that produced in another.

*M10. Effect of mastitis upon milk quality and composition.* P. A. DOWNS, University of Nebraska.

In this study no attempt was made to study the whole milking but rather the complete secretion of the individual quarters of the udder. This was accomplished by the use of both milking machine equipment as well

as a four-compartment pail so that the milk from each quarter could be kept separate without altering the regular milking process. The preliminary study of 36 different animals in this manner indicates that there is great variation in the amount of milk produced by the different quarters of the udder at a given milking.

The tabulation of results obtained from cows that have had no garget history indicates that the hind quarters consistently gave more milk than the fore quarters at the same milking. Fresh cows apparently showed greater variation than did cows further along in lactation. When the production of the hind quarters was taken as 100 per cent, it was found that the average of eight animals in normal lactation was 57.2 per cent for the right front and 56.2 per cent for the left front quarters. The study of 26 animals, which have had garget history, showed great variation in production. Percentage production of front and rear quarters showed reduction as great as 90 per cent when compared with production of the corresponding quarter at the same milking. The amount of reduction apparently depending upon the extent of the disease and condition of the udder.

While the fat content of the milk varied greatly from quarter to quarter, no definite trends could be observed.

Studies of the production of a cow being milked three times a day were made for 30 consecutive days. One quarter showing an active case of mastitis (garget) showed marked reduction in milk flow and in general a lower percentage of fat in the milk produced. The production of the other quarters showed marked variation in production from milking to milking. Extreme variations of 5.5 pounds to 1.5 pounds at the following milking and 6.7 pounds to 2 pounds were noted. The variation seemed to be consistent in all quarters for that particular milking. The percentage of fat varied greatly, showing a trend toward the highest percentage of fat in the largest amount of milk usually following a milking of low production with low percentage of fat.

The quality of milk as shown by appearance, pH (Brom Thymol Blue Test), leucocyte count on original milk, and presence of streptococci in incubated samples, showed great irregularities during the 30-day period. The quarter having active mastitis showed definite changes in pH varying from 7.2 to 5.5 which seemed to occur in waves from one extreme to the other while in the most active stages, and then remaining between 6.5 and 7.2 while in the less active stage. In the other quarters a variation of pH from 6.5 to 6.7 was noted but was not consistent from milking to milking. The active quarter showed high leucocyte count and streptococci in the original milk. The number of streptococci present during the most active stage increased and decreased periodically as did the leucocyte count. In the less active stage the streptococci count was less than 10,000 cc. but they were found periodically in the incubated samples.

In the quarters that were infected but showed no signs of garget there was no consistent result either of leucocyte count or streptococci count in the incubated samples. Neither the Brom Thymol Blue Test nor the streptococci count in incubated samples showed the presence of the infection at all milkings.

*M11. The relation of mastitis to the rennet coagulability and strength of milk.* H. H. SOMMER AND HELENE MATSEN, University of Wisconsin.

Curd strength and rennet coagulation studies were made on the milk samples from the cows of the University of Wisconsin herd as segregated into normal and mastitis groups on the basis of diagnoses made independent of this study. The tests that were used in the diagnosis were: udder manipulations to detect abnormalities, strip cup test, chloride content by direct titration, brom cresol purple test, catalase test, and plate colony count on blood agar.

The curd strength was determined by a modification of the Hill test. The coagulation time with rennet was determined at 30° C. with rennet extract added at the rate of 1 part to 2,500 parts of milk. The reaction of the milk samples was determined by the quinhydrone electrode at 35° C.

A preliminary survey of the milk from the individual cows in the two groups gave the following results on curd strength:

GROUP	NO. OF COWS	NO. OF TESTS	AVERAGE CURD STRENGTH IN GRAMS
Normal	31	388	48.56
Mastitis	15	191	39.17

A smaller number of rennet coagulation tests were made. The following figures give the average results for rennet coagulation and for curd strength on the same samples:

GROUP	NO. OF COWS	NO. OF TESTS	AVE. CURD STRENGTH (Grams)	AVE. RENNET COAG. (Minutes)
Normal . . . . .	30	94	46.81	7.56
Mastitis . . . . .	15	51	37.87	10.61

The cows with infected udders were then milked to keep the milk from the quarters separate and comparisons were made between the normal and mastitis samples from the same udder. The following average results were obtained in this comparison:

QUARTER	NO. OF COWS	NO. OF COMPARISONS	AVE. CURD STRENGTH (Grams)	AVE. RENNET COAG. (Minutes)
Normal	16	19	44.82	9.53
Mastitis	16	19	24.28	41.69

One case was encountered in which the milk from the infected quarters was slightly more acid than the milk from the normal quarters. In this case the milk from the infected quarters coagulated more rapidly with rennet than the milk from the normal quarters.

A similar study was made of the milk from the individual quarters of 40 normal cows. The variations in curd strength and rennet coagulation between the milks from the four quarters of the same udder were comparatively slight. The average curd strength in this study by individual quarters was 53.51 grams; the average rennet coagulation time, 7.37 minutes.

The conclusion is reached that mastitis, even when sufficiently mild so that the milk is still normal to casual observation, usually causes a significant decrease in the curd strength and an increase in the rennet coagulation time of the milk.

*M12. Structural changes occurring in casein during cheese ripening as shown by X-ray diffraction studies.* S. L. TUCKEY, H. A. RUEHE, AND G. L. CLARK, University of Illinois.

When an X-ray diffraction pattern of freshly made cheese curd is secured, two halos are registered on the photographic film. By measuring to the centers of greatest intensity, spacings of approximately 7.3 A. U. and 4.6 A. U. are obtained. After the cheese has ripened for approximately a week at 50° F., the halo of larger diameter gives way to two lines which indicate spacings of 4.6 A. U. and 4.2 A. U. After two weeks, the small halo becomes defined as two lines of 7.3 A. U. and 9.6 A. U. At four weeks, all lines are more sharply defined and also new lines at 3.04 A. U. and 2.34 A. U. have appeared. The material that diffracts the X-rays may be one or more amino acids. X-ray diffraction patterns of 20 amino acids are being made to answer that question. The material is not tyrosine as indicated by a comparison of patterns and calculated spacings. The material cannot be extracted by either hot water or ethyl alcohol.

The property of the cheese curd to become stringy so that it can be stretched slightly begins during the matting process and increases during ripening. When the cheese curd is treated with hot water, the curd can be pulled into long threads two or three feet in length. This physical change does not bring about an internal rearrangement of diffracting units such as occurs when gelatin, hair, rubber, etc., are stretched. This property

appears to be more comparable to a plastic flow rather than an actual stretching. Hence, this indicates the ultimate structure of cheese casein is radically different from the above materials, with the diffracting units having no strong polar bonds and also, not having a micellular structure such as gelatin. If the cheese curd had the property of becoming oriented upon stretching, the size of the unit cell could be calculated. This would give us further information as to the molecular weight of casein and paracasein.

*M13. Effects of time and temperature of holding milk heat-treated at various temperatures upon its subsequent coagulation by rennet.*  
MILTON E. POWELL, University of Minnesota.

Recent work at this Experiment Station has shown that calcium caseinate-colloidal calcium phosphate sols exhibit a hysteresis-like effect, similar to that of natural milk, when heat-treated and coagulated with rennet at various intervals after heating. Continuing the study of this phenomenon the effects of the time and temperature of holding heat-treated skimmilks were observed.

Fresh skim milk was heat-treated at 65° C., 75° C. and 85° C. for 30 minutes; a portion of each lot of milk was held at the heating temperature while other portions were promptly cooled to 35° C. and 5° C. and held at the respective temperatures. Fifty cc. samples from each of the above portions were coagulated with rennet at various intervals after the initial heating period. All samples were coagulated at 35° C. in an electrically heated and controlled water bath, one cc. of a three per cent rennet solution being added to each 50 cc. for coagulation; each test was made in duplicate. The water bath holds a series of glass coagulation tubes fitted with glass-tipped rubber tubing that extends through the bottom of the bath, a fine stream of milk can be released into a beaker where coagulation is observed on the sides.

The data show that when fresh skim milk is heat-treated at 65° C. for 30 minutes, cooled to 35° C. and rennet added immediately its time of coagulation is slightly less than that of the raw milk. After holding the heat-treated milk for 2½ hours at 35° C. only a slight decrease in coagulability resulted, the coagulation time equalling that of the raw milk. If the heat-treated milk is held either at 5° C. or 65° C. after the initial heating period a slight but gradual loss of coagulability occurs as rennet addition is delayed; the effects are essentially the same at either temperature.

When the fresh milk is heat-treated at 75° C. for 30 minutes and coagulated immediately at 35° C. the coagulation time is twice that of the raw milk. Prolonged holding of this heat-treated milk at 5° C., 35° C. or 75° C.

before the addition of rennet causes further decreases in coagulability, the greatest retardation occurring at 75° C. and the least at 35° C.

Increasing the heating temperature to 85° C. causes similar but more drastic effects on coagulability. The coagulation time of a sample immediately after heat-treating at 85° C. and cooling to 35° C. was four times that of the raw milk. Holding the heat-treated milk for 1½ hours at 5° C. or 85° C. after the initial heating period retarded the coagulability so much that samples failed to coagulate within one hour after addition of the rennet. That portion held at 35° C. required nearly 45 minutes to coagulate or 10 times as long as the unheated control.

The change in pH is negligible, barely measureable, regardless of the temperature or time of heating and holding.

*M14. Determinations of copper in sugar condensed milk and some relations between the copper content and off flavor in strawberry ice cream.* HAROLD L. LINK, HARRY J. KONEN, AND L. A. BAUMANN, Xavier University and French-Bauer Inc., Cincinnati.

From various methods given for the determination of minute quantities of copper, the potassium ethyl xanthate and sodium-diethyl-dithio-carbamate methods were chosen as the most practical. Considering time, simplicity, accuracy, cost, and adaptability these methods are the best in view of the observed data. Ninety-five per cent copper was recovered from control samples by the carbamate reagent, while the xanthate recovered 100.6 per cent. In the analysis of sugar condensed milk, the carbamate recovered 95.6 per cent copper and the xanthate 92.5 per cent. Using these two methods interchangeably throughout more than one hundred determinations, a general average of 97.5 per cent copper was recovered. The writers consider concentration cells as a means of determining minute quantities of copper well worth attention.

Five samples of strawberry ice cream were prepared with copper added in equal portions ranging from 0.28 to 1.39 p. p. m. The sample with the largest amount of added copper developed the off flavor in 16 days while the rest did not become distinctly off until 51 days. The ice cream with no added copper developed the off flavor at the end of 65 days.

Since the samples with added copper developed the off flavor much sooner than the sample with no added copper, it can be definitely stated that copper with time is a factor in the production of the off flavor. The writers suggest that since this off flavor has been described as occurring only during the winter season, future work be done to determine whether or not the quick turn-over during the summer months is responsible for the absence of the off flavor. Perhaps the change in diet of the cows is a factor to be considered, for the quality of milk is positively known to change with a change in diet.

**M15.** *The application of the Minnesota Babcock method to the testing of ice cream, concentrated milk and chocolate milk.* L. M. THURSTON AND W. CARSON BROWN, West Virginia University.

Readings of Minnesota Method results for identical samples of plain ice cream, ice cream mix and evaporated milk were found to vary with (1) digestion temperature, (2) shaking procedure during digestion and (3) length of digestion period. Digestion at 180–185° F. yielded higher results than digestion in a boiling water bath. Vigorous shaking of the mixtures in the test bottles during digestion, especially during the early stages of digestion, gave higher results than were obtained by not shaking or by shaking gently. Digestion periods in boiling water longer than 20 minutes gave lower results than periods of less than 20 minutes and the lowering of the results was progressive as the time beyond 20 minutes was increased.

Based on the above observations the following method of procedure was found to give accurate results for plain ice cream, ice cream mix and evaporated milk:

Weigh 9 grams of prepared sample each into two 20 per cent ice cream test bottles. Add 15 cc. of Minnesota Reagent. Digest 12 to 15 minutes in a gently boiling water bath, having the bottles in a rack held at least 2 inches above the bottom of the bath. Shake the tests vigorously at the time when at least half the contents of the bottle have turned dark brown (usually about 2½ minutes after placing them in the water bath). Shake vigorously again about 1 minute later. (Note: Some care may be necessary when starting to shake the bottles the second time as the "Petrohol" in the reagent may boil off through the neck of the bottle taking with it some of the mixture). Place the tests in a centrifuge and centrifuge them for one-half minute at the speed used for regular Babcock tests. Add hot water (130–140° F.) to float the butterfat well up into the neck of the test bottle. Centrifuge for one-half minute. Place the tests in a water bath at 133–137° F. and leave for 5 minutes. Just before reading each test allow colored reading fluid to flow gently onto the surface of the fat column. Hold the bottles in a level position and read as one would read a Babcock cream test. To secure accurate readings apply divider points to the smooth side of the bottle neck. When adjusting the lower point of the dividers keep the eye on a level with that point and when adjusting the upper point raise the eye level accordingly. Average the duplicate determinations.

The Minnesota Method does not yield accurate results for chocolate ice cream and chocolate milk. Good results can be obtained with the method for sweetened condensed milk if great care is observed in following out the procedure.

**M16.** *The standardization of the Borden Body Flow Meter for determining the apparent viscosity of cream.* J. C. HENING, New York Agricultural Experiment Station, Geneva.

Due to a demand from production men of milk plants for a simple method of estimating cream body, a simple efflux instrument called the Borden Body Flow Meter was developed by Nair and Mook.

Since this instrument is coming into general use in milk plants for determining the apparent viscosity of cream it seemed desirable to compare results obtained with it with results obtained by using a standard instrument such as the Mac Michael viscometer.

The results of comparative tests of the two instruments with sucrose solutions ranging from 20 to 65 per cent sucrose and cream ranging in fat content from 30 to 52.5 per cent are reported in this paper. The viscosity determinations of the sucrose solutions were made at 20° C. and those of the cream were made at 15.6° C.

The viscosities in centipoises for sucrose solutions as determined by the Mac Michael viscometer when plotted against the seconds as determined by the Borden Body Flow Meter give a straight line relationship passing through zero which shows that the Borden Body Flow Meter gives correct results throughout the entire range for a true solution.

When plotting the results for cream, in a similar manner, the creams containing 30, 35 and 40 per cent fat show practically a straight line relationship but for creams of higher fat content the relationship is shown by a curved line.

For creams of higher viscosity the Borden Body Flow Meter consistently gave results which were higher than those obtained with the Mac Michael viscometer. However, results on very viscous creams may be transposed to approximate centipoises by the standardization data secured on cream thereby making it possible for those using the Flow Meter to compare their results quite accurately with data reported in centipoises.

**M17.** *Judging sweet cream.* J. H. NAIR, D. E. MOOK, AND R. S. FLEMING, Borden's Research Laboratories, Syracuse, N. Y.

In the past, little attention has been given to judging methods for sweet fluid cream intended for immediate distribution. While the "Score Card for Milk and Cream" which has been adopted by the U. S. Department of Agriculture may be quite satisfactory for market milk, it scarcely covers all characteristics which should be considered in judging the quality of sweet cream.

The bulk of fluid cream sales occurs in urban centers. Statistics compiled to several of the large eastern markets show that at least 40 per cent of the total milk volume used in these markets is distributed in the form of fluid cream. This may not hold strictly throughout the U. S. but it is evident

that fluid cream occupies, both from the angle of dollar sales and the total volume of milk entering into it, a very high place in the economics of fluid milk distribution. It appears that a score card which would properly measure the quality of fluid cream should be quite valuable to the industry and the public in evaluating the quality of the product. Such a score card should include those characteristics which are factors in consumer acceptance.

A scoring plan is suggested for general use which embodies these ideas. This plan includes scoring of flavor, body, cream plug and serum separation, color, sediment and container. Methods of determining viscosity and whipping quality are discussed, and standards of quality are suggested under each heading of the scoring plan. It is proposed that the Association appoint a committee to formulate a suitable universal score card for cream only, which may eventually serve as a basis for grading market cream.

*M18. Some factors affecting the properties of whipped cream.* W. S. MUELLER, M. J. MACK, AND H. G. LINDQUIST, Massachusetts State College.

The variable factors studied and described in this paper are:

1. Rate of cooling after pasteurization.
2. Homogenization and milling of the cream.
3. Added serum solids.
  - a. Condensed skim milk.
  - b. Skim milk powder.
4. Addition of various stabilizers.
  - a. Gelatin.
  - b. Dairloid.
  - c. Kraftogen.
  - d. Sodium caseinate.
  - e. Vegetable gelatin.
5. Effect of sugar.
  - a. Amount.
  - b. Time of adding.
6. Effect of feed.

The cream was whipped with the mechanical whipper as described in the JOURNAL OF DAIRY SCIENCE, XVIII, No. 3, 177. By connecting a sensitive wattmeter with the whipper motor it was possible to determine the relative stiffness of the cream throughout the whipping process. All samples of cream were whipped to their maximum stiffness. Tables are presented showing the maximum stiffness, as measured in watts, time to obtain maximum stiffness, average watt increase per second of whipping, overrun, amount of drain after 24 hours, per cent of fat in drain and relative viscosity of cream after aging. Graphs present the stiffness of the whipped cream at regular intervals of 10 or 5 seconds after whipping was started.

The keeping quality of the whipped cream when stored in pint ice cream cartons at 38° F. was noted, as this is of interest to the dairyman selling whipped cream.

*M19. Frequency of the flavor defects in milk.* EARL WEAVER, E. I. FOUTS, AND P. C. MCGILLIARD, Oklahoma A. and M. College.

A milk flavor study in progress since August 1, 1933, has provided 928 milk samples which have been scored. The samples are obtained from Jersey cows in the college herd. The cows receive special care on the days of sampling to prevent feed flavor in the milk. A sample from each cow is collected at the evening milking on each Tuesday. It is cooled and held at 40° F. till the next morning at 8:00 when it is warmed to 90° F. and scored.

Scoring is usually done by four or five staff members. The scoring is aided by a chart as described in the JOURNAL OF DAIRY SCIENCE, Vol. XVIII, No. 1. The chart lists 18 defects that may be noted in milk flavor.

To date 928 samples have been scored. If we consider one judge's score on one sample as an observation there have been 4,262 such observations. In only 9.95 per cent of the observations did a judge fail to note a flavor defect. These were the only samples scored excellent. In 1.5 per cent of the observations the judge was unable to identify the flavor he detected.

The following enumeration ranks the 18 defects according to the frequency with which each was used. The figure accompanying each defect gives the percentage of all the observations in which the particular defect was the one noted. They are: (1) feed 19.73 per cent, (2) cowy 15.49, (3) rancid 10.39, (4) stale 8.47, (5) salty 4.67, (6) flat 4.65, (7) sweet .87, (8) bitter .45, (9) metallic .38, (10) weedy .38, (11) oxidized .35, (12) sharp .35, (13) nutty .33, (14) cooked .31, (15) watered .21, (16) acidy .09, (17) musty .02 and (18) disinfectant .02 per cent.

These defects used alone account for 67.23 per cent of all observations. Frequently two defects were noted in a sample, in 10 cases three defects were noted and in 1 case there were four.

For instance, "feed" along with other criticisms was noted in 7.60 per cent of the observations. "Cow" was associated with other defects in 7.65 per cent of the cases. "Stale" was so noted in 6.98 per cent of the cases and "rancid" in 5.16 per cent.

Despite the effort to prevent feed flavors they were the most frequent offenders. Cowiness was nearly as frequent. The "rancidity" is the most serious of all because it is often so offensive as to make the milk inedible. Its frequency is of concern. The "stale" flavor is likewise serious.

These four defects—feed, cowy, rancid, stale—were observed as the sole criticism of the milk in 54.08 per cent of the observations. The other 14 defects occurring singly account for but 12.15 per cent.

Various combinations of flavor defects constitute 21.32 per cent of the

observations and as stated above in 1.5 per cent of the cases the defect was not identified while in 9.95 per cent no criticism was offered.

*M20. Effects of feeds on oxidized flavors in pasteurized milk.* ED. PREWITT AND E. H. PARFITT, Purdue University.

Fourteen cows in the Purdue University dairy herd were fed rations composed of commonly used concentrates which included ground white and yellow corn, ground oats, ground soybeans, wheat bran, soybean oil, soybean oil meal, linseed oil meal, dried brewers grains, and gluten feed to determine the effect of these rations upon the development of oxidized flavor in pasteurized milk. All of the cows were fed the same roughages, silage and alfalfa hay.

The milk samples were collected immediately after milking from aluminum pails, pasteurized in Pyrex flasks by heating to 63° C. and holding for 30 minutes, then cooled to 4.4° C. Oxidized flavor failed to develop in any of the samples of milk from cows fed these rations when the samples were stored at 4.4° C. for 72 hours.

The effect of the various rations on retarding the development of oxidized flavor in milk to which had been added minute quantities of copper compounds was also determined. The results indicate that the rations containing soybean oil either direct or in the unprocessed beans, had a tendency to produce milk which upon holding developed less degree oxidized flavor than did the milk from cows fed other rations.

*M21. Methods of studying feed effects on the physical properties of butterfat.* WILLIS D. GALLUP, J. I. KEITH, AND A. H. KUHLMAN, Oklahoma A. & M. College.

Apparatus and routine procedure have been developed to study the influence of feed, particularly cottonseed meal, on the physical properties of butterfat. Although chemical methods furnish information about the true nature of butterfat, they often fail to describe accurately such properties as body, texture, and hardness. Only under carefully controlled conditions have the usual chemical constants been correlated with physical properties.

In the tests described, measurements are made of the hardness of butterfat by determining the weight of mercury required to force a plunger 5 mm. in diameter through a disc of butterfat 6 mm. in thickness. The determinations are made at temperatures between 18.2° and 23° C. at intervals of 0.6°. At 20° C. the hardness of "herd" and cottonseed meal butterfat samples expressed in cc. of mercury is approximately 9 and 23 respectively. Measurements are also made of hardness at 0° C. by determining the depth of penetration of a standard rod into a sample of the hardened fat.

Melting time is measured at temperatures between 45° and 75° C. at intervals of 5°. A determination is made of the time required for 25 cc. of

butterfat in a standardized tube to melt and form a clear liquid when transferred from an ice bath to a water bath maintained at the desired temperature. At 45° C. the melting time of different samples varies between 14 and 20 minutes.

Congealing time is determined on a 5 cc. sample of fat placed in one arm of a Y-shaped test tube which contains a free-moving small steel ball. The tube is transferred from a water bath held at 40° C. to one held at 20° C. and rocked back and forth until the sample solidifies sufficiently to hold the ball in a fixed position.

*M22. Effect of a heavy cottonseed meal ration on milk and butter.* J. I. KEITH, A. H. KUHLMAN, EARL WEAVER, AND W. D. GALLUP, Oklahoma A. & M. College.

In this series of studies the mixed milk from five Jerseys on a heavy cottonseed meal ration was compared to the mixed milk from five Jerseys on a normal herd ration. For sake of simplicity the milk or milk products from the former are designated as "cottonseed meal" and from the latter as "normal."

The results obtained from scoring the two milks show that the flavor score of the cottonseed meal milk was slightly higher than that of the normal milk. While the cottonseed meal milk was often criticized as having a slightly flat flavor, this was not as serious as the feed flavor which was more common in the normal milk.

There was no difference in the exhaustiveness of separation of the milks or the exhaustiveness of churning of the creams.

When the cottonseed meal cream was churned at the same temperature as normal cream it required about 55 per cent longer time to churn. In order to make the churning time of each kind of cream the same it was necessary to churn the cottonseed meal cream at about 6° F. higher temperature. For correct working of the butter it was necessary to use a correspondingly higher temperature of wash water.

When butter color was used in the normal cream at the rate of one ounce per hundred pounds of fat it was necessary to use 1.75 ounces of color in the cottonseed meal cream.

When working the butter it required 124 revolutions of the churn to incorporate the desired amount of moisture, whereas 99 revolutions of the churn incorporated a little more than the desired amount of moisture in the normal butter.

The cottonseed meal butter scored about .7 of a point lower than the normal butter, due mostly to its somewhat gummy body.

*M23. The detection and control of bovine mastitis.* G. J. HUCKER, New York Agricultural Experiment Station, Geneva.

A study of a long series of samples of milk from mastitis-infected cattle which subsequently have been available for post-mortem examination, has

indicated that milk containing more than 500,000 cells per cc. always signifies a pathological condition in the udder. In addition, long chain streptococci are not found in udders which are free from any traces of fibrosis or induration. Udders free from all pathological changes do not discharge long chain streptococci or a great number of leucocytes.

It has been found that there is a definite correlation between the various laboratory and barn tests which are now available to detect udder infections. From the standpoint of examination of quarter samples, the least effective is the strip cup, while certain of the more complicated laboratory tests will detect abnormalities when only traces of infection are present in the herd. From a practical standpoint, the brom thymol blue test appears to be the most adaptable, while in the laboratory the examination of incubated samples, the use of Burri slants, and the leucocyte count, are to be especially recommended.

It is exceedingly difficult to eliminate mastitis from dairy herds but it is possible to bring it under reasonable control. This may be brought about by a dairymen adhering to a general routine, as follows:

1. Test each quarter in the herd for infection by either the strip cup or brom thymol blue test.
2. Segregate as soon as possible at one end of the milking line and milk last all cows reacting to either of these tests as well as all cows giving milk that is watery, thick or showing any evidence of infection.
3. All replacements in the herd should be tested (brom thymol blue) before being added to the herd. If replacement is dry, the advice of a competent veterinarian should be secured.
4. See that all cows are milked dry at each milking. If a machine is used, strip dry promptly.
5. Watch all cows with injured quarters carefully for infection.
6. Reasonable sanitary precautions should be carried out in the management of the herd.

Milk from suspected cows should not be milked on the floor.

Although no definite data are available on the effect of feeding infected milk to calves, it is advisable not to use mastitis infected milk for calf feed unless it has been brought to a boil.

7. If trouble is still experienced after using the above methods, and if a more stringent method of detection of infected quarters than outlined above is desired, get in touch with your milk inspection laboratory or a competent veterinarian for a more intensive study of the herd.

*M24. Studies on aseptically drawn milk from Bang's disease positive and Bang's disease negative cows.* H. B. MORRISON AND F. E. HULL, University of Kentucky.

A study was made of milk drawn aseptically from 57 cows in the Kentucky Agricultural Experiment Station dairy herd from 1931 to 1934. Determinations made were brom thymol blue reaction, leucocyte count, bacterial count, agglutination test for *Brucella abortus* and titratable

acidity. One hundred and thirty-two samples were studied from 13 cows in the so-called positive herd of which all but one were reactors to the agglutination test for Bang's abortion. Four hundred and forty-nine samples were secured from 44 cows which made up the so-called negative herd. None of the cows in the negative herd were reactors to the agglutination test for Bang's abortion.

Of the samples from the positive herd 57 or 43.2 per cent gave reactions to the brom thymol blue test while from the negative herd only 19 or 4.2 per cent showed reactions.

The leucocyte counts of the samples from the positive cows averaged 1,291,000 per cc. as compared with 441,000 per cc. for those from the negative cows. The average leucocyte count of the samples reacting to the brom thymol blue test was 2,326,000 per cc. and for the samples normal to this test the average was 395,000 per cc. The average leucocyte count of all samples was 639,000 per cc.

Of the 132 milk samples from the positive herd 51 or 38.6 per cent gave reactions to the agglutination test. Twenty of these samples also reacted to the brom thymol blue test. Sixty-eight samples from this group reacted to one of these tests, but not to the other while 44 samples gave negative reactions to both the agglutination and brom thymol blue tests.

*M25. Bitter flavor in cheddar cheese made from pasteurized milk.* C. A. PHILLIPS, University of California.

This is a progress report on work being conducted to attempt to determine the relation of certain lactic cultures to the development of bitter flavor in cheddar cheese made from pasteurized milk, and also to determine the relation of acidity at the different stages of the making and curing process to the development of the bitter flavor.

Twenty-two lots of cheese were made from milk of average quality, selected at the University of Wisconsin\* creamery platform, and pasteurized in a vertical removable coil pasteurizer at 62° C. to 64° C. for 30 minutes. Some lots were cooled to 5° C. and were held over night, while other lots were cooled to 31° C. and were made into cheese immediately.

Three different cultures of *Streptococcus lactis* were used. Two of these were commercial starters, and the third was isolated from a prize-winning cheddar cheese. Two cultures of lactobacilli, obtained from the United States Department of Agriculture, Washington, D. C., were used to supplement the one of the commercial cultures in some lots.

The cheese was made according to the regular cheddar cheese process, using 300 pounds of milk for each lot. The rate of acid development was varied in the different makes. The curing was done at an average temperature of 7° C.

\* The work to date on this project has been conducted at the University of Wisconsin, Madison, Wisconsin.

Hydrogen-ion concentration measurements were made on the curd and cheese at regular intervals. The cheese was graded each month during the curing process.

The rate of acid development in milk and in the curd during the making process was found to vary with the different cultures of *Streptococcus lactis*. With all of the cultures, however, when the pH value of the cheese was 4.90 at 4 days, bitter flavor subsequently developed. It also appeared in a majority of the lots of pH 4.95 and in a part of the cheese of pH 5.00, but not in the cheese of higher pH values.

In cheese having a pH value at 4 days of 5.00, an improvement in flavor was noticed when one of the commercial starters was supplemented with the lactobacilli. The other two lactic starters were not tried with the lactobacilli.

Off flavors of the fermented type developed in some of the cheese having pH values at 4 days of from 5.15 to 5.25. In these the salt content was lower than in the cheese which did not develop the off flavors, indicating the possibility of a critical salt concentration for this type of cheese. No relation was found, however, between bitterness and salt concentration within the limits of salt used.

*M26. Study of a gassy defect in cream cheese.* W. J. CORBETT, W. C. FRAZIER, AND W. V. PRICE, University of Wisconsin.

Manufacturers of cream cheese sometimes encounter serious difficulty from gas development in cheese packed under vacuum in 6 ounce cocktail glasses. The defective cheese has a yeasty fermented taste and odor. The gas accumulates in small bubbles which are sometimes filled with whey. Eventually the gas developed releases the vacuum and may actually lift the covers and cause the contents of the glass to spill over the sides. The defect is commonly noticed during the summer months, and seems to occur more frequently in cheese flavored with pimientos, pineapple or relish than in the plain cheese.

Five typical samples of defective cheese were plated on dextrose nutrient agar, potato nutrient agar, acidulated dextrose nutrient agar, and acidulated potato nutrient agar. Colonies were picked into glucose nutrient broth and those cultures which produced gas were replated until pure cultures were isolated as shown by the gram-stain.

Lactose fermenting yeasts of the genus *Saccharomyces* were isolated from the cheese. These yeasts reproduced the defect when they were inoculated into sterile cheese and packed under vacuum. Addition of sucrose or flavoring materials such as pimiento, pineapple, or relish increased the amounts of gas produced. None of the aerobic or anaerobic bacteria which were isolated from the cheese were able to produce the defect.

Preliminary thermal death point studies have been made with several

of the yeasts which showed that the yeasts were readily destroyed at pasteurization temperatures.

Market samples of two local and two nationally advertised brands of cream cheese which are sold in Madison were studied bacteriologically over a period of 10 weeks beginning in March, 1935.

Plate and microscopic counts were made to show the total numbers of organisms in the cheese. Number of yeasts and molds were determined. Samples of cheese were incubated in sealed test tubes and the volume of gas produced at 28° C. was measured. In all cases where gas was produced yeasts were present in large numbers. The bulk cheese had lower yeast and mold counts than the packaged product.

*M27. Varieties of the genus Oospora found in cream and butter.* C. M. SORESENSEN, Purdue University.

Eight cultures of *Oospora* were isolated from samples of neutralized sour cream butter. The morphology and cultural characteristics of these cultures were studied and described as to their effect on three carbohydrate broths, limus milk, dextrose gelatine, nile-blue sulphate butterfat agar, skim milk agar and potato dextrose tartaric acid agar. Observations on their behavior and effect on cream under varying conditions of acidity and temperature in pure culture and in mixed culture were also recorded.

Notable differences in proteolysis, lipolysis, growth rates, temperature relations, thermal death points, and colony characteristics were found within the eight cultures isolated.

*M28. The disappearance of acetylmethylcarbinol and diacetyl in butter cultures.* G. I. STAHLY, M. B. MICHAELIAN, C. H. WERKMAN, AND B. W. HAMMER, Iowa State College.

The importance of acetylmethylcarbinol and diacetyl in butter cultures is indicated by the fact that cultures having a satisfactory flavor and aroma contain relatively large amounts of these materials, while cultures lacking in flavor and aroma contain only small amounts or none. The maximum production of acetylmethylcarbinol and diacetyl in a culture is ordinarily followed by a decrease, the rate of which depends on a number of factors.

Various investigators have considered that acetylmethylcarbinol may be reduced to 2,3-butylene glycol by certain bacteria, and this suggests that the disappearance of acetylmethylcarbinol and diacetyl in a butter culture may be due to such a reduction. This suggestion was tested in a series of trials with pure cultures of the citric acid fermenting streptococci normally present in butter cultures and with butter cultures themselves.

When tomato bouillon was inoculated with one of the citric acid fermenting streptococci, the organism allowed to increase in numbers at 21°

C., and acetylmethylcarbinol or diacetyl then added, there was a rapid decrease in the added reagent and a corresponding increase in 2, 3-butylene glycol. Reduction was less rapid in cultures in which the pH was reduced to about 4.0 with sulfuric acid than in unacidified cultures. When milk was used as a medium instead of tomato juice, reduction of added acetylmethylcarbinol or diacetyl also occurred but often at a somewhat slower rate.

In butter cultures held at 21° C. decreases in the amounts of acetylmethylcarbinol and diacetyl were accompanied by corresponding increases in 2, 3-butylene glycol. When cultures were held at about 0° C. there were slow decreases in acetylmethylcarbinol and diacetyl and comparable increases in 2, 3-butylene glycol. The relatively rapid decreases in acetylmethylcarbinol and diacetyl which occurred in neutralized cultures at 21° C. and the relatively slow decreases in such cultures at 0° C. were also accompanied by compensating increases in 2, 3-butylene glycol.

*M29. A study of Escherichia-Aerobacter organisms in pasteurized milk.*  
JOSEPH L. MINKIN AND L. H. BURGWALD, Ohio State University.

Gassy fermentation in pasteurized milk is undesirable. The usual cause is *Escherichia-Aerobacter* organisms. Their presence in pasteurized milk is considered as an indication of inefficient pasteurization, or subsequent contamination after pasteurization.

Presumptive fermentation tests were run on more than 350 commercially pasteurized milk samples obtained from more than 100 pasteurizing plants.

Approximately forty per cent of the pasteurized samples gave gas in the presumptive test. Partially confirmed tests were made on E. M. B. and Endo media.

Heat tests were run on the *Escherichia* and *Aerobacter* forms from the confirmed tests. About 35.0 per cent of the organisms tested were able to resist pasteurization temperatures.

More definite figures will be available when the last group of samples are put through the various tests.

This work indicates that certain of the *Escherichia-Aerobacter* organisms are able to live through pasteurization. Previous workers have shown this also. This paper gives an indication of the relative importance of these heat resisting organisms. Their presence will make it more difficult to evaluate results of fermentation tests made on pasteurized milk.

*M30. Observations on yeasts causing gas in sweetened condensed milk.*  
H. C. OLSON AND B. W. HAMMER, Iowa State College.

Two types of yeasts were isolated from gassy sweetened condensed milk and studied in detail. An oval type, which was identified as *Torula lactis*-

*condensi*, was isolated from all of the eight samples of gassy milk examined, while a spherical type was found in two of them. The name *Torula globosa* is suggested for the spherical type, which apparently is identical with the spherical yeasts isolated from sweetened condensed milk by various investigators. The differences between *T. lactis-condensi* and *T. globosa* involve primarily morphology, extent of growth on solid media in the absence of fermentable material, and growth temperatures.

From the reports of previous investigators and from the information obtained in this study it appears that gas formation in sweetened condensed milk is commonly due to *T. lactis-condensi* or to *T. lactis-condensi* and *T. globosa* growing together, and that when the two species are growing together *T. lactis-condensi* is the more numerous. A pure culture of *T. globosa* inoculated into sweetened condensed milk produced gas, and one investigator found only this species in a sample of milk that had blown under commercial conditions.

*M31. Standard laboratory methods for the control of dairy products.*

ROBERT S. BREED, New York Agricultural Experiment Station,  
Geneva.

A brief outline will be given of the progress that has been made during the past year in preparing standard laboratory methods that are useful in the official control of dairy products. This will include a discussion of efforts that are being made in other countries to develop more uniform laboratory procedures in this field. The matter is one of interest, not only to the industry but also to the men who are responsible for official control work.

Further research is needed along several lines, some of which will be indicated briefly.

*M32. Acidity in the manufacture of cream cheese. Z. D. ROUNDY AND  
W. V. PRICE, University of Wisconsin.*

Approximately 100 lots of cream cheese were made by the cooked-curd process in which a 16 per cent cream was pasteurized at 145° F. for 30 minutes, homogenized at 1500 pounds pressure per square inch at 120° F. using the single stage Manton-Gaulin, and coagulated by the action of rennet and lactic starter. The results of modifying the conditions of coagulation indicated that this type of cheese does not react like either the neufchatel cheese, which it displaced commercially, or the rennet-curd cottage cheese, which is commonly coagulated in an identical manner.

Rennet was varied from 0 cc. to 28 cc. per thousand pounds of cream and failed to alter appreciably the quality of the cheese.

Starter inoculations varying in amounts from 0.1 to 25 per cent were used. Large inoculations shortened the setting time. There was no ad-

vantage in using amounts greater than 5 per cent because (1) the rate of draining was retarded which more than compensated for the reduction in the time of setting. The retardation was due to the addition to the mix of unhomogenized milk in the form of starter. (2) There was danger of imparting to the cheese a cooked flavor due to the high heat treatment to which the milk for starter was subjected. (3) There was danger of obtaining a high acid or starter flavor in the finished cheese.

Temperatures higher than 72° F. but not higher than approximately 90° F. shortened the setting time. At 100° F. the time of incubation was increased. At 110° F. the *Streptococcus lactis* organisms did not produce enough acid to bring about coagulation.

These results indicated that the cheese making operations might successfully be shortened without injuring the quality of the cheese. Further experiments showed that by using 5 per cent of starter, 3 cc. of rennet per 1000 pounds of cream and setting at 90° F. it was possible to complete the cheese making process in one day.

The success of these modifications depends upon the exact control, either by titration or electrometric measurements, of the acidity of the mix at the time of cutting or breaking. A certain development of acid is necessary to impart to the cheese the characteristic smooth, waxy, consistency. When, at the time of cutting, the acidity of the cream is too low, the cheese has a tendency to be coarse and grainy. On the other hand, an excessive acid development retards the rate of draining and may cause a high-acid flavor. The cheese is almost invariably dry and crumbly when the acidity of the cream at the time of cutting is less than pH 5.0. When the acidity at cutting is greater than pH 4.8, the cheese is usually very smooth in texture but acid in flavor.

The acidity of the mix at cutting influences the loss of fat in the whey. Excessive fat losses occurred when the pH was higher than 5.0. These losses ranged from 1 to 2 per cent depending upon the acidity of the mix. At pH 4.85 or lower the loss of fat in the whey should not exceed one tenth of one per cent by the extraction test.

*M33. The manufacture of a soft cheese of the Bel Paese type.* ROBERT R. FARRAR, Bureau of Dairy Industry, United States Department of Agriculture.

A method of manufacturing a soft cheese of the Italian Bel Paese type has been developed. The cheese has a characteristic, mild, clean, lactic flavor, a soft and waxy texture, and good slicing and spreading qualities.

Numerous factors which might be expected to influence the quality of the cheese were investigated. It was found that a uniform product of high quality resulted when the following factors were closely controlled: composition, including percentages of moisture, fat, and salt; kind, activity,

and amount of starter; setting temperature; amount of rennet as dependent upon the coagulability of the milk; size and firmness of curd particles; rate and amount of drainage and length of drainage period; size, type, and shape of forms; temperatures of manufacturing and curing rooms; time and method of salting; and time of wrapping.

The cheese is made from fresh whole milk with the addition of lactic starter and rennet. The setting temperature is 40° to 42° C. The ripening temperature is 3° to 7° C., the relative humidity 80 to 90 per cent. The cheeses are wrapped at about 20 days and are fully cured at 5 to 6 weeks. Artificial refrigeration is necessary. The yield is about 11 to 14 per cent. Actual sales have shown a very good consumer demand.

*M34. Experiments with canned cheddar cheese.* E. L. REICHART, University of Nebraska.

The canning of cheddar cheese and its commercial adaptation was developed by L. A. Rogers, H. L. Wilson and associates of the U. S. Department of Agriculture. The original outline for handling cheese in this manner was published in the spring of 1934 and several commercial companies have since tried this method of merchandising cheddar cheese. Our experiments were undertaken in an attempt to determine the desirability of using this method under Nebraska conditions. As normal temperatures and atmospheric conditions are rather undesirable in this state for proper ripening in the average cheese factory, it seemed that the possibility of placing the cheese into sealed cans immediately at the time it was made and ripening it under controlled temperature conditions in the can would afford quite decided advantages.

The general method of packaging the cheese outlined by H. L. Wilson was followed in these experiments. All cheese was made from pasteurized whole milk, varying in fat content from 3.3 to 4 per cent. Two per cent starter was added to the milk and 3½ to 4 ounces of rennet per 1000 pounds of milk was used. Otherwise, the method of making was that commonly used in making a Wisconsin style cheddar cheese. The curd was pressed in a specially built horn-shaped hoop (made by Damrow Brothers), pressed for 1 to 2 hours, dressed, and pressed overnight. The cheese was then cut in 13-ounce portions, wrapped in parchment paper and canned in a specially constructed 12-ounce vented can designed and manufactured by the Continental Can Company. These cans were then stored at different temperatures to determine the effect of temperature on the ripening of the cheese. These cans were examined after 20, 30, 60, and 120 days and scored.

**RESULTS.**—These results are entirely preliminary, covering a relatively small number of batches, but the following results are quite apparent. Cheese made from poor-quality milk, placed in cans, and ripened at rela-

tively high temperatures (60–70° F.) makes a lower scoring final product than when placed in daisies or 5-pound prints and ripened at the usual ripening temperatures for these styles. Cheese made from good-quality, milk, properly made, canned, and ripened at 60–75° F. will make a materially higher scoring cheese after a considerably shorter ripening period than cheese ordinarily made in daisies or prints and ripened in the usual manner. It is quite possible, if a reasonably good supply of milk is available and manufacturing methods are reasonably carefully followed, to make cheese scoring from 92 to 95 points consistently. Thirty- to forty-day ripening periods at 60–70° F. are sufficiently long to produce a satisfactorily ripened cheese for ordinary trade demands. This cheese is much better ripened than the average longhorn or daisies being marketed in this section of the country. Of 32 batches made to date, from milk just as it is received daily without any effort being made to select milk more carefully than for ordinary cheese making purposes, we find that after 30 days aging at 70° F., 18 batches scored 92 or over, 5 scored 90 or over, and 9 scored under 90. It seems from preliminary trials to be quite possible to adapt this method of packing cheese to a number of varieties and modifications of ordinary cheddar cheese. The commercial possibilities of manufacturing cheese under this method seem to be quite good.

*M35. The vitamin A content of sour cream butter, sweet cream butter and margarine.* I. L. HATHAWAY AND H. P. DAVIS, University of Nebraska.

Nineteen samples of margarine were obtained from Illinois, Ohio, and Nebraska, analyzed chemically, and their vitamin A content compared to that of sour cream butter or sweet cream butter. The fat content of the butter samples varied from 80.2 to 81.5 per cent while the fat content of the margarine samples varied from 78.3 to 89.2 per cent. Only two of the nineteen samples of margarine contained enough vitamin A to permit the rats to grow even when the margarines were fed in amounts ten times as great as were the butters. One of these samples caused an average gain of ten grams per rat and the other sample an average gain of twenty-four grams per rat. From these results it was evident that these samples of margarine were very poor sources of vitamin A when compared to butter.

*M36. Technic, examination and reporting extraneous matter in butter.* B. E. HORRALL, Purdue University.

The activity of the Federal Food and Drug Administration demanding that butter be clean has led the Dairy Industry to scrutinize butter from a different viewpoint than it has heretofore—namely, the extraneous matter content.

The butter manufacturers of Indiana and neighboring states cooperat-

ing with the Purdue Dairy Department started a project last August in which the manufacturers agreed to send in one or more pounds of butter each month for a period of one year. The extraneous matter content of 700 samples of butter sent in by the cooperators has been analyzed. A technic and examination of butter for extraneous matter has been developed.

The method suggested by the Government for filtering butter has been modified to meet conditions existing in the sour cream areas. The wide-field low-power microscope with  $25\times$  and  $75\times$  magnifications was found the best to identify the extraneous matter on the filter paper. The kind, source, and angle of light was found to be essential in aiding to find and to identify the extraneous matter on the filter pads.

*M37. Notes on the national cream quality improvement campaign.* M. E. PARKER, Sealtest System Laboratories, Inc., Cleveland.

A progress report of the part played by the creamery butter industry in the development and furtherance of the National Campaign for Improvement in the quality of cream supplies used in the manufacture of creamery butter.

*M38. Comparative efficiency of electrically operated tanks versus ice in the cooling of milk.* J. H. FRANSEN, Massachusetts State College.

Whether the tank is of home construction or specially manufactured, it is essential that it be of ample capacity; roughly speaking, when filled with cans to full capacity, there should still be room for twice as much water and ice as milk.

If the tank is of home construction, there should be at least 3 or 4 inches of cork or its equivalent, and this of course must be protected against moisture. Such insulation saves more than its cost in saved refrigeration in one season.

Electric cooling of milk is entirely practical. The machines we have tried are convenient, reliable, and, if well adjusted, economical. We have no information as to the probable service cost and annual depreciation charge.

Electric milk cooling tanks are a distinct labor-saving device and we believe that within a comparatively few years nearly all dairymen will wish to be equipped with electric devices for cooling milk.

Results of our experiments would indicate that the water in the tank should always be as high as the milk line. This can be accomplished by the addition of more water to make up for the absence of cans during the time it is run with part load, or the same results can be accomplished by providing some arrangement whereby weighted empty cans can be placed

in the tank so as to raise the water line to the height of the milk in the cans. They are more sanitary than natural ice.

*M39. Frozen brines as refrigerants for ice cream in cabinets and shipping containers.* H. H. SOMMER, University of Wisconsin.

This brief study was undertaken to assist a manufacturer of ice cream delivery truck bodies in the selection of a suitable brine to supply the refrigeration. A survey of physico-chemical tables revealed the following data with respect to cryohydric points for various salts arranged in the order of temperature:

SALT	CONCENTRATION		CRYOHYDRIC POINT	
	Salt	Water	°C	°F
BaCl <sub>2</sub>	22.5	77.5	- 7.8	+17.96
MnSO <sub>4</sub>	32.2	67.8	-10.5	+13.10
Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub>	30.0	70.0	-11.0	+12.20
KCl	19.75	80.25	-11.1	+12.02
K <sub>2</sub> CrO <sub>4</sub>	36.6	63.4	-11.3	+11.66
NH <sub>4</sub> Cl	18.6	81.4	-15.8	+ 3.56
Ca(NO <sub>3</sub> ) <sub>2</sub>	35	65	-16.0	+ 3.20
NH <sub>4</sub> NO <sub>3</sub>	41.2	58.8	-17.35	+ 0.77
NaNO <sub>3</sub>	37	63	-18.5	- 1.30
SrCl <sub>2</sub>	26.0	74.0	-18.7	- 1.66
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	38.3	61.7	-19.05	- 2.29
NaCl	22.4	77.6	-21.2	- 6.16
Cu(NO <sub>3</sub> ) <sub>2</sub>	36	64	-24.0	-11.20
NaBr	40.3	59.7	-28.0	-18.4
NaOH	19	81	-28.0	-18.4
Mg(NO <sub>3</sub> ) <sub>2</sub>	34.6	65.4	-29.0	-20.2
MgCl <sub>2</sub>	21.6	78.4	-33.6	-28.48
K <sub>2</sub> CO <sub>3</sub>	39.5	60.5	-36.5	-33.7
CaCl <sub>2</sub>	29.8	70.2	-55.0	-67.0

A number of these salts were tested for use in brine cartridges by placing the brine in a No. 2 sanitary can. A half inch copper tube (3½ inches long) was closed at the lower end, the under end was flanged and soldered into place through a hole in the can cover. After the cover had been sealed onto the filled can, this arrangement provided a brine cartridge with the copper tube serving as a recess to the approximate center of the can for the insertion of a thermometer. After the brine had been frozen in a hardening room a suitable thermometer was inserted with alcohol used as the conducting medium. The melting curves that were obtained for various brines in this manner showed that the zones through which the temperature was approximately constant closely approached the cryohydric points as listed above.

In these melting tests absolute constancy of temperature was not attained as might be expected on the basis of theoretical considerations. This is attributed mainly to the lack of agitation, permitting some settling out of the precipitated salt from the partially melted brine. Traces of impurities may also be partly responsible.

*M40. An experimental ice cream freezing unit.* J. I. KEITH AND C. W. RINK, Oklahoma A. & M. College.

When doing research work on ice cream it is often difficult to do any extensive series of freezings under accurately controlled conditions if only one freezer is used. Furthermore, a considerable loss is incurred in disposing of large quantities of ice cream where an adequate market is not available. To overcome these difficulties at Oklahoma A. and M. College a small freezing unit has been designed.

This paper describes the construction and operation of the unit which consists essentially of four one-gallon freezers set in a brine box.

The box is built of one-inch cypress wood mortised and glued at the joints and held together by outside stay-bolts. The inside dimensions of the box are 24"  $\times$  24"  $\times$  11 $\frac{3}{4}$ ".

Small bevel pinions are substituted for the hand cranks on the freezer drive shafts. These are all turned toward the center of the box where they are engaged by the same bevel driving gear. Power is furnished by a  $\frac{1}{2}$  h. p. motor.

The brine in the box is siphoned from a large brine tank and overflows through an outlet 6" from the bottom of the box. Brine temperature may be changed very quickly or may be held constant within a fraction of one degree Fahrenheit.

Any freezer may be placed in the box, started, stopped or removed while the others are running.

Holes are cut in the freezer lids to enable the operator to observe the ice cream as it freezes.

Overrun is measured by a gage without removing ice cream from the can. On duplicate batches of the same mix overrun may be controlled to within 1 per cent at any specified time interval.

The total cost of materials and construction of the entire unit was less than \$100.00.

*M41. Methods for testing condensing pans.* L. C. THOMSEN, University of Wisconsin.

The chief purpose of this paper is the presentation of a method for running tests on a vacuum pan in commercial operation. These tests were made on a collandria type, 36-inch diameter stainless steel pan which had

a heating area of 40 square feet. Data were collected on seven runs. A complete set of data are included in the results. Methods for calculating the information in the data table are presented.

Information obtained indicates the advantages of a vacuum pan for removing water from dairy products.

An efficiency of heat utilization of 92.0 to 98.8 per cent was found. The efficiency of the condenser varied from 50.7 to 72.1 per cent. Water evaporation per hour per square foot per degree Fahrenheit temperature difference varied from 0.244 to 0.493 pounds. The amount of steam based on unit evaporation necessary to evaporate one pound of water from milk varied from 1.04 to 1.13 pounds. The amount of condensing water per pound of vapor from the milk varied from 17.5 to 20.7 pounds. The overall coefficient of heat transfer varied from 248 to 497 B.t.u.

Other data are also presented.

## EXTENSION SECTION

*E1. Revision of record forms for D. H. I. A. work.* J. W. LINN, Kansas State College.

A study of answers to a questionnaire sent to every Extension Dairyman listed as in charge of D. H. I. A. work in the various states indicates that the Herd Record Book B. D. I. 5 (Revised) has incorporated most of the suggested changes.

Interest in the simplification of feed records is increasing. There is also a trend toward the keeping of more complete herd records that will give the total amount of feed consumed by the herd, assist in proving sires, and make it possible for the tester to render services heretofore impossible.

A permanent herd record book is being used in a few states. This assists in proving sires, and in studying the efficiency of individual cows and the breeding efficiency of the herd. The development of a uniform permanent herd record book should undoubtedly be the next step in improving D. H. I. work.

*E5. A yearly association program.* A. B. NYSTROM, Bureau of Dairy Industry, U. S. Department of Agriculture.

Every dairy herd-improvement association should have a definite program of work to follow each year for the benefit of members, testers, and the county agent. This program should show what is to be done, how, and by whom it is to be done, and when the work must be finished. This will insure a larger volume of work each day, month, and year. The needs of the farmers and their willingness to cooperate must determine how extensive the program should be. But even the simplest program should be carefully planned to obtain maximum results in its execution.

The kind of program to undertake should be determined by a survey. One type of survey form is presented. This can be simple or elaborate to suit the needs and capabilities of the tester and members. In no case, however, will a verbal survey suffice. A record must be made of conditions found and filed for later reference. The survey should first of all include questions on matters pertaining to the dairy herd, its breeding and management, number and kinds of animals. It might also include statements about the buildings, the cropping system, condition of land as regards fertility, and the farmer's interest in cash crops.

A tester or an association will accomplish more by marking down a definite goal to be reached during the month and year. The goal should be possible of attainment, yet high enough to require extra effort to reach.

A list of 43 possible goals is given. It is not presumed that any association would attempt to reach more than a few of these goals in any given year, but there is sufficient variation in the list to suggest goals that will touch all phases of the farm enterprise. A suggested plan of work for a sample association with goals and calendar of work is also presented.

#### WHO WOULD BENEFIT

The greatest benefit from such a program would come to the members of the association. But all of the farmers in the neighborhood would benefit through the meetings, tours and exhibits held by association members to which everyone in the community would be invited. Dairy herd-improvement association work should be so planned and conducted as to make each member of the association a demonstrator of some good practices for the benefit of others, as well as himself.

The tester would be benefited by the program, because he would be able to plan his work more systematically and to get more work done during the year. If he anticipates reaching a definite goal by a certain date, he becomes more enthusiastic about the work he is doing and this tends to lighten his burden.

The county agent would benefit through having a definite check on what is going on in his county. He should welcome the plan because it will make it possible for him to carry out with least effort a well rounded dairy program, based on the actual needs of the farmers.

*E9. Five-session dairy feeding school.* C. L. BLACKMAN, Ohio State University.

#### PURPOSE

As the name suggests, this school is divided into five sessions and deals primarily with dairy feeding problems. It is designed also to stimulate a broad interest in the science of dairying and to show the importance of this enterprise to human welfare.

An effort is made not only to give as much fundamental science as seems necessary but to briefly trace its development in order to acquaint farmers with the fact that our knowledge is constantly changing. This is done in the hope that farmers will not only be more receptive to new facts but also be on the lookout for them.

An outline of each discussion is furnished to those attending the school. This makes it possible for the instructor to cover more material and gives those attending something which they may use for study between meetings.

The general plan is to hold one session each week in a county, at the same time and place, over a period of five weeks. A good proportion of those attending the school are present at all five meetings.

## SUBJECT MATTER

*Lesson I* deals briefly with the early history of dairying and the introduction of cattle into this country. This lesson also deals with the early and recent discoveries and investigations which brought about dairy progress. Some facts are given as to the relative importance of dairying in the nation and state.

The last part of the lesson deals with investigations which show that high production is necessary for economical production.

*Lesson II* gives the reason for the guaranteed analyses of feeds and explains briefly how such analyses are made. It illustrates the meaning of digestible nutrients by giving the data from a digestion trial. This lesson also explains the methods of absorption and the use to which the various nutrients are put.

*Lesson III* deals with characteristics of a desirable ration, explaining the reason for these requirements by reference to specific experiments. Much of this lesson is devoted to a discussion of the common feeds and their particular characteristics. Special emphasis is placed on the value of high quality roughage and considerable information is given on how to produce good roughage. (The Agronomy Dept. of the O. S. U. has co-operated in furnishing much of these data.) Hay and grain samples are used as demonstration material during this discussion.

*Lesson IV* deals with the development of feeding standards and explains their value in formulating rations. This lesson is supplemented by a bulletin on the general subject of dairy cattle feeding.

Much of the fourth discussion is devoted to the actually balancing of rations for members. Members are assisted in making their own calculations. It is hoped that after this lesson those attending may be able to calculate rations themselves.

*Lesson V* is something of a review of previous lessons and an application of what has been previously discussed to practical problems of feeding the dry cow, the calf, and the cow in heavy milk. Some time is also devoted to the subject of management as it relates to the control and elimination of the common diseases, such as tuberculosis, abortion, mastitis, milk fever, etc. The brom thymol blue test is demonstrated at this meeting.

The five-session feeding school has given a great opportunity to get better acquainted with many dairymen and their problems. It furnishes a means of giving more technical information than would otherwise be possible. Occasionally farmers change practices during the course and report results. These reports are the basis for some stimulating discussions.

Schools of this character could be improved and made more interesting by using more demonstration material.

*E12. Making dairymen out of 4-H club members.* DWIGHT M. SEATH, Kansas State College.

State extension programs, for the most part, have failed to make dairymen of 4-H members enrolled in dairy projects.

Much of this is due to an over-emphasis of the less essential feature of the program. Too much stress has been placed on the phase which deals with the type of dairy animals, while too little attention has been given to the teaching of principles which deal with the science of breeding, feeding, and management of profitable dairy herds.

One method of lessening the emphasis on type in the showing of 4-H Club calves is to award the club members, for their skill in fitting and showing, premiums equal to those made for the excellence of the club animals they exhibit.

More stress also needs to be given to the third-year dairy club work. Definite premiums, preferably in the form of educational trips or scholarships, to those who excel in the application of sound dairy practices in the handling and the keeping of records on the producing cow will help stimulate this program.

There is a need for more junior projects which deal with breeding better dairy cattle. Junior bull clubs, which are being used in a few states, have partially filled the need. Such work needs to be popularized so that there will be an equal chance for recognized achievement in this field as in other phases of dairy club work. The same is true of projects pertaining to record keeping and the management of dairy farms.

*E13. Listing cow testing association proved sires and permanent herd records.* EARL N. SHULTZ, Iowa State College.

The publishing of data for sires proved by means of cow testing association records is a problem which has confronted Extension Dairymen in recent years. To be of the greatest value the information in the daughter and dam comparisons should be available not only to the owner of the sire proved but to all dairymen interested in improving their herds by intelligent breeding methods. The amount of work required of the tester in securing the information should also be considered, as there is a limit to what can be expected of these men.

Breed associations and several states have published proved sire data, but each one has followed a different plan. The object of this study was to consider the various plans and offer recommendations as to the data which should be published.

A questionnaire was sent to all extension specialists in charge of cow testing associations and dairy cattle breeding work of the various states to get what these men thought most important in the publishing of sire data.

From the results of the study of the various plans and the replies to the questionnaires, the following recommendations were made:

The list should include the name and number of all sires with 5 or more unselected, tested daughters from tested dams. The date of birth of the sire should be listed, the name of his owner and address, the date proved and whether alive or dead when proved. The production records calculated to maturity should be reported for—

1. All daughters with lactation records.
2. All daughters with lactation records which can be compared with the records of their dams.
3. All dams of above daughters.

The average increase or decrease should also be given, but no index calculated. With the information at hand, any preferred index can be calculated.

#### PERMANENT HERD RECORDS

Cow testing association members who have tested continuously for a number of years find that the information contained in a half dozen or more herd books is not easy to use. A compact, readily available, loose-leaf permanent record system is in demand by these dairymen. Several states have started such a system.

A study of various types of permanent herd records was made and a suggested form prepared. This form was a loose-leaf system on sheets  $8\frac{1}{2}'' \times 11''$  in size. Information for each cow, such as her production, reproduction, health and records of her progeny, is to be listed on one sheet. For such a record system to be workable, some scheme of identifying the animals in the herd is necessary, such as by ear tags or tattoo marks.

*E15. Analysis of dairy herd improvement association proved sire records.*

E. J. PERRY, State University of New Jersey.

The Bureau of Dairy Industry is making a valuable contribution to the Better Sires Program of the country through the tabulations which are now being prepared with such dispatch. The data recorded on the sheets returned to the state colleges are splendid but considerably more information is needed if worthy proved sires and their offspring, particularly the sons, are to create the interest which they merit. These additional facts must be gathered by the testers and state extension men and used along with the figures from the Bureau before the sires can be fully appraised.

Many factors may enter into the average production of a set of daughters. Were any culled or sold for milkers in the proving period? How many records were made, and which ones were used? This question has particular reference to the dams since most bulls are proved by the 2-year-old lactation record of the daughters. What is the bull's index? What were the health conditions when the records of the daughters and

dams were made? Did a change in feeding methods or other environmental factors play a part? What kind of type is the bull siring? These are the questions that are being asked about proved sires today. Are we able to answer them satisfactorily?

The sire proving program should be a step in the larger program of cooperative breeding. At present most of the sires that are being proved are owned by individuals. It seems very difficult to arrange for an exchange of sires where there is no joint ownership. When the health program of a group of herds is on the same basis bull associations or breeding circles are the practical solution to the problem of extending the usefulness of good proved sires. More data are needed on the results secured over a period of years by proved sires and by well-managed bull associations. Denmark has such data which show that the average production of the herds that have been enrolled in both the Breeding Societies and Cow testing associations over a period of 30 years is 23 per cent higher than the production of those herds that have been in testing associations but not in bull associations (Breeding Societies). Figures of like character are needed from our bull associations and from herds where meritorious sires have been identified through the performance of their daughters. Data such as this should be presented periodically to all who are enrolled in the sire project. They should be urged to keep a permanent herd record book.

How best to secure this needed supplementary information rests with the extension service of each state but it is highly desirable that the methods and results be as nearly uniform as possible.

*E16. Sons of proved sires.* D. L. FOURT, University of Idaho.

It is a recognized fact that the use of meritorious proved sires is the surest method of improving dairy herds through breeding. Unfortunately the number of meritorious proved sires is limited.

Preliminary studies indicate that sons of meritorious proved sires can be used with greater assurance of success than sons of untried sires.

Records indicate that less than one-half of all sires increase production, while preliminary studies of Idaho records indicate that approximately two-thirds of the sons of meritorious proved sires increase production.

The use of sons of meritorious proved sires should be emphasized to a greater extent in the dairy extension program. Continuous herd testing is essential. Young bulls should be selected on the transmitting ability of their sires and dams rather than on the producing ability of the dams. Proper interpretation of pedigrees should be emphasized, and padded pedigrees deflated. The adoption of pedigrees showing the transmitting ability of sires and dams should be encouraged.

*E17. Standardized lactation records for reporting dam and daughter comparisons in D. H. I. A.* J. F. KENDRICK, Bureau of Dairy Industry, U. S. D. A.

The type of lactation record to use in dairy herd-improvement associations as a basis for selecting cows and evaluating the transmitting ability of sires has been given considerable attention during recent years. Investigators generally agree that the type of record which has high repeatability is the best measure of the production capacity of the cow.

Variation found in lactation records may be largely attributed to six general causes: (1) Differences in management and feeding, (2) differences in length of lactation periods, (3) differences in number of times cow is milked each day, (4) differences in the degree of influence of gestation, (5) differences in ages, and (6) differences in inheritance of cows.

It is impractical if not impossible at the present time to correct for variations caused by differences in management and feeding and in the inheritance of cows. As a result of this study, a standardized 305-day M. E. (mature equivalent) record has been devised to correct for variation caused by differences in length of lactation periods, differences in number of times cow is milked each day, differences in the influence of gestation and differences in age. The standardized 305-day M. E. record has higher repeatability than lactation records consisting of the first 305 days' or the first 365 days' production.

The standardized 305-day M. E. record will measure production capacity and evaluate the transmitting ability of sires with greater accuracy than has heretofore been possible.

## PROBLEMS AND METHODS OF INSTRUCTION

### 11. *A basic curriculum for an agricultural college.* H. P. DAVIS, University of Nebraska.

#### OBJECTIVES

A curriculum to function satisfactorily must be built upon the carefully worked out ideals and purposes of the college as determined by the faculty. These objectives cover four divisions, namely, cultural, physical, moral, and professional and may be summarized as follows: To provide a broad general education so as to help students understand their environment and appreciate the broad field of human knowledge and at the same time develop their personalities; to teach a sound appreciation of health and its maintenance; to inculcate an understanding of moral and ethical principles; and to insure adequate professional training in agricultural science. All these objectives having as their purpose the training of an educated, right thinking, independent and useful citizen.

#### COURSE SELECTION

A curriculum should be built up by the faculty to insure the carrying out of the objectives by testing each part of each course. Courses are divided into required and elective. In general, the required courses are designed to give a survey of the field and the orientation of that field to other subjects.

#### REQUIRED COURSES

The required courses insure that the student has had a broad background before being allowed to specialize in a particular field.

<i>Kind of Course</i>	<i>Semester Credits</i>	
English—4 courses		10
Orientation		1
Physical Education—2 courses		2
*Military Science—4 courses		4
Biological Sciences		13
General Biology	4	
General Botany	3	
General Zoology	3	
General Bacteriology	3	
Physical and Exact Sciences		12*
Chemistry—2 courses	8	
Physics	2	
Mathematics	2	

\* Prescribed by the Board of Regents.

\* At the University of Nebraska, only 8 credits in chemistry are required.

<i>Kind of Course</i>		<i>Semester Credits</i>
<b>Social Sciences</b>		12
Political Science	3	
Economics—2 courses	6	
Psychology or Sociology	3	
<b>Agricultural Sciences—survey courses</b>		28
Agricultural Engineering	3	
Agronomy—2 courses (soils & crops)	6	
Animal Husbandry	3	
Dairy Husbandry	3	
Entomology	3	
Horticulture	3	
Poultry Husbandry	2	
Rural Economics	3	
Vocational Education	2	
<b>Agricultural Sciences—elective</b>		12
<b>Total</b>		94

## ELECTIVE COURSES

A total of 40 credits are required in agricultural sciences of which 12 may be elective. Thus out of 125 credits, 82 are fixed courses, 12 are electives in agricultural science and the balance may be selected in any course in the university.

*12. Certain suggestions for a course of study for those majoring in dairy industry.* J. H. FIANDSEN, Massachusetts State College.

(No abstract submitted.)

*13. Building a course in dairy husbandry.* H. P. DAVIS, University of Nebraska.

The building of a course in Dairy Husbandry involves many problems for the instructor and a thorough understanding and appreciation of the ideals back of the college curriculum. Study must be made of the position of the proposed course in the curriculum, as for instance, whether it appears the first year or at some later time. The complexity of the course and the subject matter presented will depend to a considerable extent upon when the proposed course is given.

Courses may be divided into four general types, namely, (1) survey, (2) basic principles, (3) factual information, and (4) development of skills and techniques. Any particular course may represent one or any combination of two or more. The question of whether the course is to be required and for what class of students must also be settled. A required course, if the only one required in a department, will be of a different make-up than if it is the first of two or more required courses. Required

courses for the general student body should be of a different character than those required for a professional group. A required course for all students given the first year would likely be largely of a survey character, while a required course for Dairy Husbandry majors in the junior year, for example, would be more factual, present more basic principles and probably attempt to teach skills and techniques. In general, elective courses will be designed to have a student appeal.

The type of instruction or method of presentation has a marked effect upon the construction of the course, and a choice of methods must be made before the actual start is made. The first procedure is to formulate the objectives. These should be both inclusive and exclusive and should be the criteria for measuring material to be presented. The material bearing on the subject is next surveyed and that part eliminated that does not carry out the objectives. The next step is the division of the material into class periods and the making of such adjustments as are necessary. With the course material roughly allocated to class periods, then the class period or laboratory outline is prepared for each class meeting. This outline clearly states the purpose or purposes to be accomplished, asks significant questions, and gives the references. Finally, each outline is checked with other courses both within and without the department to determine if there is duplication. A successful course depends to a large extent upon logical methods of preparation, which will likely insure proper balance and integration.

14. *Frequent quizzes in teaching dairy elements.* E. L. FOUTS AND J. I. KEITH, Oklahoma A. and M. College.

Teaching the elements of dairying to classes of over one hundred students, made up chiefly of freshmen, presents one of the most difficult problems found in the teaching schedule of the instructor in dairying. The instructor is obliged to teach the students how to study, to emphasize the need for study and to leave with the students some knowledge of the subject of dairying.

Observations taken during the presentation of dairy elements over a period of ten years have led the writers to draw the following conclusions:

A text-book is absolutely essential for this type of course.

A ten minute written quiz at the beginning of practically every theory period encourages systematic study and promotes interest in the subject.

Prompt return of graded papers and the immediate posting of all daily grades on a bulletin board creates unusual interest and acts as an incentive for the poorer students to improve their standing.

The entire course should be planned in advance of the first meeting of the class and the sequence of the work in lecture and laboratory given careful consideration.

The use of many exhibits and demonstrations illustrating important points has been found to add interest and to aid in teaching the subject to the best advantage.

Maintaining the interest of the students in the work being given is the keynote of success in teaching this type of course.

15. *Adjusting dairy instruction to the needs of the state.* C. E. WYLIE,  
University of Tennessee

The dairy instruction program in any state university should be related to the conditions in the state and the needs of its students.

CONDITIONS IN STATE—TENNESSEE

- I. Cattle.
  1. Ninety-three per cent purebred dairy cattle are Jerseys.
  2. Ninety-five per cent of grade dairy cattle are Jerseys.
  3. The average test of milk is 4.4 per cent fat.
  4. Less than  $\frac{1}{2}$  of 1 per cent of cattle tested react to T. B.
- II. Climate
  1. Annual rainfall 60 inches.
  2. Many large springs, 58-60° F
  3. Long growing and grazing seasons.
  4. Outdoor comfort.
- III. Marketing products—Diversified.
  1. Thirty-one per cent market milk
  2. Forty-three per cent cream for creamery butter.
  3. Twenty-five per cent milk for manufacturing purposes.
  4. Farm butter—not included above.
- IV. Students.
  1. Large percentage live on farms.
  2. Many have some dairy farm experience.
  3. Few have manufacturing experience.
- V. Program and policy.

Not to increase the number of cattle or dairymen but to improve quality of products and the efficiency of producing them.
- VI. Demand for trained men.

Type of positions available.

INSTRUCTION

- I. Cattle—4 herds.
  1. College—Registered Jerseys and Holsteins for instruction, demonstration, and research.
  2. Junior College—Registered Jerseys for instruction and demonstration.

3. Two sub-stations—Registered Jerseys for experimental and demonstration purposes.

## II. College Creamery.

1. Milk and cream purchased from over 200 dairymen.
2. Products—creamery butter, ice cream, cultured milks, chocolate milk, pasteurized and raw milk, cottage, American and cream cheese.
3. Labor—mostly students.

## III. Courses and instruction.

1. Four-year course in agriculture, specializing in dairying.
2. Four-year cooperative course—students alternating by terms working and going to school.
3. Short Course—for experienced persons making a life-work of dairying.
  - a. Dairy farmers.
  - b. Dairy manufacturing workers.
  - c. Vocational teachers.
  - d. Nutrition and health workers.
4. One year dairy course.  
Less than college grade and no college credit.
5. Graduate students.

## 16. *The integrated course of study in agriculture.* A. M. FIELD, University of Minnesota.

The primary objective for teaching agriculture in the secondary schools is to provide rural youth with the knowledges, skills, attitudes, and habits most needed for farming and rural life in a modern era. The agriculture course is based on the interests and needs of the students and it is regarded as an integral part of their farm life.

Agriculture as a mode of life is made up of a series of inter-related activities which together constitute the total pattern of successful farming. The former plan of teaching crops, animal husbandry, soils, mechanics and farm management in separate yearly units has given way to an integrated course of study. The program for instruction in agriculture is organized the way a farmer farms. The course of study is regarded as a continuous program of planning, study, and instruction extending over three or more years. The problems of the home farms serve as the basis for determining content. The problems for each year are selected on the basis of the interests, abilities and needs of the students. The subject matter is developed on the enterprise basis and is arranged to progress in sequence of difficulty within the enterprise as the students pass from one year to the next and as they gain in maturity, experience and power of understanding. Individualized instruction and practical application on the home farms are important features of the teaching plan.

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## ABSTRACTS OF PAPERS PRESENTED AT ANNUAL MEETING, SUPPLEMENTARY TO JULY NUMBER

*E3. Farm Account Work Through D. H. I. A. Testing.* A. J. CRAMER, Wisconsin College of Agriculture.

Wisconsin dairymen in Dairy Herd Improvement Association work have made farm account record keeping play a big part in building their farm income the past three years. No matter how good a dairy may be it cannot make up for the losses caused by poor crop production.

The farm account records call for—

1. Inventories of land, buildings, machinery, livestock, feeds, and miscellaneous supplies, taken at beginning and end of the year.
2. All farm expenses and receipts.
3. Production records of crops and livestock crop acre yields and the production of different classes of livestock.

Most testers spend 20–30 minutes each month with a member helping him to make entries in his farm account record book.

The problems which must be met are:

1. The records too often are not finished when sent in.
2. Some testers are not farm management minded.
3. Some inaccuracies in keeping records were found.
4. The new simplified book has encouraged many farmers to keep farm account records with their dairy herd records.

In 1932, there were 157 members completing books in 45 associations. Now there are 2,048 books being kept with the help of 81 testers. This is a 1200% increase over the first year the work was started.

Six of the most important factors on dairy farms of Wisconsin are:

- |                                     |   |
|-------------------------------------|---|
| 1. Number of crop acres.            | 4. Per cent of crop land in alfalfa.                |
| 2. Production of butterfat per cow. | 5. Livestock returns per \$1.00 worth of feed used. |
| 3. Crop yields per acre.            | 6. Diversity of farm income.                        |

Wisconsin Farm Accounts Records were first kept in 1920 with one tester named Donald Mitchell in Waukesha County, and in 1922 with a second tester named Nander Nelson in Washington County, and in 1929

with a group of 5 testers all in Pierce County who kept records on 101 farms. Then in 1932 a special effort was made by our present Dean Christensen of the College of Agriculture who appointed a committee to consider the advisability of combining the functions of the two units of the Farm Management Department and the Dairy Records Department. The result was the office of Farm Accounts and Dairy Records. We are now starting the third year of record work with this combined office.

The first farm account record book which is discarded had a page-distribution of items by enterprises. This included inventories, purchases, sales, breeding records and management records, bills payable and receivable, all labor costs, feed crop costs, personal and household expenses, products supplied the family from the farm, etc. There were so many records that it confused the farmer and he did not wish to keep it.

The new book is put up in the form of a column distribution of receipts and expenses, with inventories and crop production records at one place in the book. We have left out half of the forms which were in the older book.

Concerning the office set-up, there are four specialists for the dairy work and two for the farm management work. There is one full-time clerk who works on the records while 5 other clerks help where work is to be done.

The record books are supplied to the cooperators (D. H. I. A. members) free of charge by the College of Agriculture; other people are charged 10 cents a copy.

The testers are given training along farm management lines each year through their short course training and through one or two 3-day schools. These schools familiarize the tester with the possible results to be obtained in keeping farm account records. Usually one day is spent going over the account book with the testers showing them how items are entered. Each tester, when hired, understands that his work consists of helping with the dairy records, and in keeping the complete record of farm income and expenses.

The tester knows that the success of his testing work hinges around increased income to the dairyman. Often the tester can help find and remedy the weak places in the farm business where records are available. The extension specialists spend time with the tester helping him with the farm account record work.

We have started most of the books after crops are harvested, after October 1 to April 1. The books are usually started at the beginning of the association year so they may be closed when the herd records are completed. We plan to get the books back to the farmers before spring work starts. They can think over the suggestions made and make changes in their cropping system for the next year.

The question often raised concerns the ability of the tester to carry on both lines of record work. I think our testers are doing as much D. H. I. A. record keeping as testers in other states with additional Farm Account records to be kept.

With the identification of all animals, the breeding and production records necessary to get daughter-dam comparisons, etc., this farm account work is the easiest work the tester is carrying. If testers are keeping the farm account records on the farm, the monthly feed records for the individual cows may be omitted, because we will get from the farm record book the feed efficiency figure which has been used more effectively than have the feed records obtained in connection with the dairy herd work alone. Usually the dairyman culls more on production than on feed efficiency of his cows.

We are looking for a better class of tester than we have in the past. Usually the tester who has kept up his herd records also does a good job with his farm account records.

The farm account record is voluntary on the part of the farmer. Some farmers prefer to keep their own records. Some farmers are requesting this work as time goes on so that the need for testers trained along farm management lines is increasing rather than decreasing.

*E8. County feeding programs.* H. R. SEARLES, Minnesota College of Agriculture.

Our extension program is being developed more and more around programs developed by groups of interested people in the counties with the assistance of the county agents and extension workers.

Because dairying is the most important farm enterprise in many of our Minnesota counties, there developed a desire on the part of a few counties to form a county dairy committee to study the needs of the county, and to formulate a county dairy program.

The committees as they have developed are made up of representatives of every active organization in the county, both farmers and business men. In most cases the members were elected or appointed by the organization that they represented.

This county committee of 30 to 40 men spend about two days with the county agent and extension dairyman in making up their program with a sub-committee in charge of each project.

The following projects are representative: Sires and Breeding, Testing, Marketing, Consumption, Disease Control, Crops and Feeding.

These committees give the county agent a working group to take over the leadership in developing a program on their project.

Several of the sub-committees have been very active and have added tremendously to the effectiveness of the county programs. They have

assisted in maintaining cow testing associations, in better bull placement and exchanges, in surveys of consumption and advertising campaigns for increasing consumption of dairy products; have held meetings and helped with the alfalfa and better pasture problems.

What place they will occupy in the future we do not know, but they should prove a real factor in developing a local dairy extension program.

*E10. Record Keeping and Reporting in 4-H Dairy Club Work.* R. G. CONNELLY, Virginia Polytechnic Institute.

Where judiciously administered, 4-H dairy club record keeping and reporting will provide an effective extension method for teaching rural boys and girls improved methods of dairy management.

Since 4-H dairy club work is a specialized educational enterprise for rural youth, a system of record keeping on the projects seems essential to the success of that enterprise. Such a system should provide technical dairy instruction; it should instill a scientific, as well as a business, attitude in rural youth towards the problems of dairying; it should provide for experience in intelligent dairy management; and finally, it should provide club members and others a basis for measuring project progress and improving dairy farm practices.

The purposes and applicability of 4-H dairy record keeping and reporting systems are manifold and extensive. They may serve in developing general extension programs in new communities; they have served as a mainstay in support of established extension programs, when the welfare of those programs was in the balance.

Any dairy record keeping and reporting system should not serve only as a basis for making 4-H club awards. It should be a means of developing purposeful dairy teaching matter on a simple plan, pertinent to the club members project. Furthermore, it should serve to improve dairy methods and the standards of rural living.

The records essential to the development of any 4-H dairy project should be classified to make record keeping and reporting easy. Names of animals, their breed, birth dates, pedigrees, etc., are semi-permanent records and should be reported once at the beginning of the project. Records concerned with yearly inventories on all animals, breeding dates, calving, feed consumption, growth, milk and butterfat production, and cost accounts change continuously, and therefore provision should be made in the state extension office for collecting and filing these records, since they should be basically important in developing the 4-H dairy teaching program.

Under proper cooperative conditions a 4-H dairy record keeping and reporting system may be adapted to fit the extension time and personnel in most any state and still yield worth while results. At present there are at least three general 4-H dairy record systems in effect. The first

and least involved is that in which the club member furnishes the essential data on himself and his club animal for entering a 4-H dairy project. He then submits to his county agent or club leader a yearly record on his project at the close of the project year. This system, though receiving rather limited supervision, has served to open the way for other more comprehensive record keeping systems.

A second system requires the club member to keep a complete yearly record book on his project which he submits along with a yearly achievement report to one of the local or state extension workers for a rating. Usually the local leader or county agricultural agent or club agent inspect the book during the progress of the project and make suggestions, but the responsibility for keeping the record up-to-date rests squarely on the club member.

A third, rather intensive system requires the club member to keep a yearly record book similar to the present Dairy Herd Improvement Association herd book. Monthly record reports are also required. At the end of the year the record books are collected, inspected and rated as a basis for making awards and planning the 4-H dairy extension program. In this system the relationship between the club member and extension service becomes more intimate than in the other systems, since closer supervision is necessary to make the system function. This system has yielded excellent results in several states.

If the purpose of 4-H dairy record keeping and reporting are to be served, the records must be carefully analyzed and used in the 4-H teaching program. The methods of analyzing and using club record information will depend upon the facts needed in the teaching program. Usually a simple summary of the records, with a computation of averages for comparative purposes, will be enough to direct the club member's attention to his project accomplishments.

Where monthly records are received they may be used effectively in developing 4-H dairy project interest or they may be sent in a monthly 4-H club letter to the club member. Honor Rolls supplemented with timely subject matter have proved particularly effective in at least one state.

In any event all the yearly 4-H records should be collected, inspected and officially acknowledged. Each club member should also receive from the state extension office a tactfully prepared letter with regard to his year's record work to give encouragement and at the same time to call his attention to points of improvement. Certificates of achievement might be issued as an acknowledgment of satisfactory project completion and the fact that the club member has qualified to proceed to the next higher 4-H project, towards the ultimate fulfillment of the state 4-H dairy extension program.

*E18-E22. Report of the Quality Improvement and Product Consumption Committee.*

Committee members: A. J. Mann, Connecticut; C. A. Hutton, Tennessee; Fred Abbott, California; E. C. Scheidenhelm, Nebraska; A. C. Baltzer, Michigan, Chairman.

A questionnaire of 14 questions pertaining to (a) quality milk and cream production and (b) consumption of dairy products by producers and consumers was sent by the committee members to each state. Forty-two replies were returned. In general the dairy department of each state agricultural college was responsible for the replies received.

There was a unanimous opinion that the quality of both raw and finished dairy products has improved in the past few years.

The changes effected in quality cream production include the following:

First, a gradual decrease in the number of cream stations; second, the cream stations now operating are tending to improve the sanitary conditions of the stations; third, a higher per cent of low acid cream is being received; fourth, cream sediment testing is increasing; fifth, the time of cream deliveries is shortening; sixth, refrigeration as an aid in quality production is growing.

Payment for cream by quality grade is practiced generally in the Pacific coast states and inclined to become a more general practice in the middle west. Very few if any cream stations are operating in the Pacific coast states.

The activities of the federal government during recent months resulting in the confiscation of butter containing extraneous matter has been the spur to improve the quality of dairy products in the majority of states. This has not burdened the producer with more expensive equipment according to the answers and in only a few instances has confiscation barred some producers from marketing products. The unanimous opinion of the states was that it has not had any unfavorable consumption reaction. The majority of the states report that some dairy products have been condemned but the percentage is less than 1 per cent.

A decided change is occurring in the states in regard to the standard of fluid milk offered for sale. It is improving in healthfulness; first, by reducing the incidence of cattle disease—removing T. B., Bang's and mastitis' reactors; and second, in quality by stepping up of the cooling requirements and sanitary conditions under which milk is being produced. The highest quality fluid milk requirements are found first, in the eastern section of the country; second, in the Pacific coast regions. Milk is not sold by quality grade, but largely by butterfat content, according to the report from these states.

Now to summarize the quality phase of this report, the committee wishes to express the opinion that, first, quality cream production increases

as the cream stations decrease in prominence. More frequent delivery, lower acidity cream, cream more free of sediment, cream with a more desirable butterfat content, and a slightly growing tendency for payment according to quality, are all factors tending to enhance quality cream production and diminish the importance of the cream station.

Second, the efforts of the department of pure food and drugs' drive to remove from consumption channels, dairy products containing extraneous matter, has served a good purpose. The amount of product confiscated is minor but the psychology of enforcement of existing laws has had a good effect.

Third, wholesome cooperation by the producer tending to improve the health standards of his herd is lending itself to a higher standard quality product.

Fourth, there has been a growing tendency for municipalities to, first, revise and bring up to date their milk ordinances, and second, for municipalities in the past without such ordinances, to make use of stronger milk ordinances and adopt in many cases the United States Public Health Milk Ordinance for the improvement of the local milk supply.

The questionnaires bring out, fifth, a decided need for enforcement of the laws pertaining both to milk and cream production. It is not the sense of the questionnaires that more laws are needed but that stricter enforcement will be helpful.

The second part of our report concerns itself with the problem of dairy product consumption. The substance of our questionnaire points out a decided lack of, first, information and second, any organized effort by agencies within the states to enlarge the consumer purchasing and producer consumption of milk and dairy products. The Pacific coast states again seem to lead the country in this regard. The dairy council activities in this region and similar activities along the Atlantic seaboard states are outstanding.

In making recommendations designed to get sustained and enlarged consumption of dairy products by consumers, the most commonly recommended means are advertising and education.

To summarize the results of this part of our report it is the sense of this committee that a fertile field exists to enlarge consumption of milk and dairy products, first, among producers themselves; second, that consumers will respond and enlarge the consumption of dairy products by emphasizing health values, safety, economy, and flavor and quality.



# THE BACTERIOLOGY OF SWISS CHEESE

## III. THE RELATION OF ACIDITY OF STARTERS AND OF pH OF THE INTERIOR OF SWISS CHEESES TO QUALITY OF CHEESES

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It has been shown in the preceding paper of this series<sup>1</sup> that *Streptococcus thermophilus* is active in carrying on a lactic acid fermentation during the first few hours that Swiss cheese is in the press and that *Lactobacillus helveticus* or a similar lactobacillus subsequently continues the fermentation. The pH values of the interior of the cheese have been used as indication of the progress of the fermentation and in particular of the effectiveness of the two kinds of starter bacteria, the lactic streptococci and the lactobacilli.

Most of the results included in the previous paper were obtained with small cheeses. It has long been realized that results with small cheeses do not always parallel results with full sized wheels. One reason is that the interior of the larger cheeses cools more slowly than the interior of smaller cheeses. The fairly common practice of cooling the kettle contents by the addition of cool water or whey just before the curd is dipped makes the temperature of the larger cheeses more like that of the smaller cheeses during the early hours of the cheese in the press. The cheeses studied in this paper were wheels with a green weight of about 155 to 160 pounds made under factory conditions at the Grove City Creamery, Grove City, Pa.

The milk used in making the Swiss cheese had for the most part a long methylene blue reduction time. The pH of the milk in the kettle was about 6.5 or 6.6 and the titratable acidity about 0.17 per cent. The fat content of the whole milk was from 4.3 to 4.9 per cent and after standardization the kettle milk contained from 3.5 to 3.8 per cent of fat. Most of the cheeses studied were not experimental but were made for the market.

The starters used were *Streptococcus thermophilus*, C<sub>2</sub> or C<sub>3</sub> strain, *Lactobacillus helveticus*, 39a strain, and *Propionibacterium Shermanii*, No. 62 strain.

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<sup>1</sup> Frazier, W. C., Burkey, L. A., Boyer, A. J., and Sanders, G. P. The bacteriology of Swiss cheese. II. The bacteriology of Swiss cheese in the press. JOURNAL DAIRY SCIENCE 18: 373-388. 1935.

Methods of sampling and examination were as described in the preceding paper of this series.<sup>1</sup>

### RESULTS

Since a pure culture starter contains only one kind of bacterium, the acidity of the starter after incubation at a given temperature will usually indicate the stage of growth of the bacteria and may indicate the likelihood of the culture being effective at the proper time in the cheese mass. A study has been made of the starters used in the Swiss cheeses at Grove City over a period of five years. Cheeses were not made continuously over that period, and cheeses included in special experiments were not included in the study.

Starters of *L. helveticus* (39a) were grown in sterilized skim milk at 37.5° to 39° C. for twelve hours. The higher incubation temperatures were used during a period when a more heat resistant organism was needed. The relation of the titratable acidity of the 39a starter used to the final grade of 862 cheeses made is shown in Table 1. It will be noted that of cheeses

TABLE 1  
*Acidity of 39a starters in milk and grades of cheeses made with them*

ACIDITY OF 39a STARTER	NUMBER OF (CHEESES)	FANCY AND NO. 1	SPECIAL	NO 2 AND NO 3
<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
< 0.90	40	62.50	12.50	25.00
0.90-0.94	20	85.00	5.00	10.00
0.95-0.99	43	53.49	32.56	13.95
1.00-1.04	114	67.54	21.93	10.53
1.05-1.09	115	63.48	26.96	10.43
1.10-1.14	121	54.55	32.23	13.22
1.15-1.19	190	56.84	24.21	18.95
1.20-1.29	91	39.56	30.77	29.67
1.30 and over	128	39.06	39.06	21.86
All of 1.2 and over	219	39.27	35.62	25.11
All of 1.0 to 1.09	229	65.07	24.45	10.48

made with starters of 1.0 per cent acidity or over, the highest percentage of Fancy and No. 1 cheeses was made with 39a starters of about 1.0 to 1.09 per cent acid; and that results with starters with 1.1 to 1.14 per cent acidity were almost as good, although the percentage of Fancy and No. 1 cheeses decreased some and the percentage of No. 2 and No. 3 (grinder) cheeses increased. A starter acidity of 1.15 to 1.19 per cent gave a still greater percentage of No. 2 and No. 3 cheeses. When a starter of 1.2 per cent acidity

<sup>1</sup> Frazier, W. C., Burkey, L. A., Boyer, A. J., and Sanders, G. P. The bacteriology of Swiss cheese. II. The bacteriology of Swiss cheese in the press. JOURNAL DAIRY SCIENCE 18: 373-388. 1935.

or over was used, however, the quality of the cheeses was considerably poorer. There was a marked increase in percentage of No. 2 and No. 3 cheeses and a marked decrease in percentage of Fancy and No. 1 cheeses, as shown in Table 1. Of all cheeses made with starters of 1.2 per cent acidity and over, 39.27 per cent were Fancy or No. 1 cheeses and 25.11 per cent were No. 2 or No. 3 cheeses, while of all cheeses made with a starter of 1.0 to 1.09 acidity, 65.07 per cent were Fancy or No. 1 cheeses and only 10.48 per cent were No. 2 or No. 3 cheeses. The high percentage of cheeses of good quality made with starters of less than 0.9 per cent acidity as shown in Table 1 is misleading. The 40 cheeses were made in two different series. Twenty cheeses from one series graded 80 per cent Fancy and No. 1 cheeses and 20 per cent Special cheeses, but the other 20 cheeses in a different series graded 50 per cent Fancy and No. 1 cheeses, but 50 per cent No. 2 and No. 3 cheeses. In the first series the milk was good enough so that a weak 39a starter was satisfactory, but a similar starter in the second series produced cheeses half of which were of low quality.

The data lead to the conclusion that a 12-hour 39a starter in milk should have a titratable acidity of not over 1.19 per cent and not less than 1.0 per cent, preferably between 1.0 and 1.09 per cent. Experience has shown, however, that when there is difficulty in getting the 39a organism to grow properly in the cheese the use of a starter with a titratable acidity of 1.1 to 1.14 per cent is preferable.

In Tables 2 and 3 are shown results of studies of cheeses made with *Str. thermophilus* ( $C_2$  or  $C_3$ ) starters of various acidities in milk and in whey, respectively. All cheeses in which the 39a culture did not develop properly

TABLE 2  
*Acidity of  $C_3$  starters in milk and grades of cheeses made with them*

ACIDITY OF $C_3$ STARTERS—MILK	NUMBER OF CHEESES	FANCY AND NO. 1	SPECIAL	NO. 2 AND NO. 3
<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Less than 0.75	17	52.94	29.41	17.65
0.75-0.80	112	67.86	22.32	9.82
Over 0.80	30	50.00	36.67	13.33

TABLE 3  
*Acidity of  $C_3$  starters in whey and grades of cheeses made with them*

ACIDITY OF $C_3$ STARTERS—WHEY	NUMBER OF CHEESES	FANCY, GOOD AND FAIR NO. 1	POOR NO. 1	SPECIAL	NO. 2 AND NO. 3
<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Less than 0.30	39	33.33	23.08	38.46	5.13
0.30-0.33	112	41.07	22.32	33.93	2.68
0.34 and over	40	17.50	47.50	27.50	7.50

were eliminated from consideration. The data in Table 2 indicate that a titratable acidity of 0.7 to 0.75 per cent is desirable in a milk starter of *Str. thermophilus* and of 0.3 to 0.33 per cent in a whey starter.

As has been stated in a previous paper, the activity of *Str. thermophilus* during the first few hours of the cheese in the press is indicated by changes in pH during this period. The pH of the interior of the cheese three hours after dipping has been used to determine the activity of the organism. Too little or too much activity will be apt to harm the quality of the cheese. The rate of acid development in the early hours of the cheese in the press can be regulated by varying the quantity of active *Str. thermophilus* starter added to the kettle milk.

In Table 4 are shown the results of studies in which the pH of 353 cheeses was determined three hours after the curd was dipped. All cheeses

TABLE 4  
The pH of the interior of the cheeses three hours after the curd was dipped  
and grades of the cured cheeses

pH OF CHEESES— 3 HOURS	NUMBER OF CHEESES	FANCY AND NO. 1	SPECIAL	NO. 2	NO. 3— GRINDERS
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
6.10-6.19	31	64.52	25.80	6.45	3.23
6.00-6.09	131	73.28	22.90	3.82	0.00
5.90-5.99	149	61.74	29.53	5.37	3.36
5.80-5.89	42	52.38	35.71	11.91	0.00

made with a weak 39a culture have been eliminated from consideration. It will be seen that the greatest percentage of cheeses of high grade and the lowest percentage of cheeses of low grade resulted when the interior of the cheeses had a pH value of from 6.0 to 6.09 after three hours in the press. There were no No. 3 (grinder) cheeses in this group. It is interesting to note that likewise there were no No. 3 cheeses but a high percentage of No. 2 cheeses in the group with a pH of 5.8 to 5.89 after three hours. These low pH values indicate great activity by *Str. thermophilus*. Such cheeses drain very rapidly and have comparatively little growth of *L. helveticus* later in the cheese. The cheeses usually rise high in the warm cellar and are over-set, but they do not ordinarily have the defects which would put them into the grinder class.

In Table 5 are shown pH readings of samples from the interior of the cheeses eight hours after the curd has been dipped into the hoop in the press. The results indicate that a pH of 5.5 to 5.69 gave the smallest percentage of low grade cheeses, but that pH 5.7 to 5.89 was practically as satisfactory.

The pH of the interior of the cheese 21 hours after dipping is taken as an indication of the growth and activity of the lactobacillus starter (39a).

TABLE 5  
*The pH of the interior of the cheeses 8 hours after the curd was dipped  
 and grades of the cured cheeses*

pH OF CHEESES— 8 HOURS	NUMBER OF CHEESES	FANCY AND NO. 1	SPECIAL	NO. 2 AND NO. 3
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
5.30–5.49	32	28.13	34.37	37.50
5.50–5.69	30	50.00	33.33	16.67
5.70–5.89	50	56.00	24.00	20.00
5.90–6.09	38	47.37	36.31	26.31
6.10 and over	28	14.29	60.71	25.00

Table 6 shows the relation of the 21-hour pH to the final grades of the cheeses. It will be noted that the best grades of cheese were obtained when the pH values at 21 hours were 5.0 to 5.09, but that almost as good a cheese was obtained when the pH value at 21 hours was 5.1 to 5.14. When the pH is 5.2 or over the grade of the cheeses is very likely to be poor. The summary in Figure 1 of results from all cheeses with a pH of 5.2 at the end of 21 hours and over and from cheeses with less than 5.2 emphasizes the danger of a pH value of 5.2 or more after 21 hours in the press, wherein only 30.39 per cent were Fancy or No. 1 cheeses and 43.81 per cent were No. 2 or No. 3 cheeses.

A reason was sought for a grade of Fair No. 1 or better of 50 (of 249) cheeses in spite of a pH of 5.2 or over after 21 hours on the press. As is shown in Table 7 these cheeses were more alkaline during the early hours in the press than most cheeses, that is, *Str. thermophilus* was less active than usual. It will be noted that the better the grade the more alkaline was the average pH after three hours and the more acid the pH after 21 hours.

TABLE 6  
*The pH of the interior of the cheeses 21 hours after the curd was dipped  
 and grades of the cured cheeses*

pH OF CHEESES— 21 HOURS	NUMBER OF CHEESES	FANCY AND NO. 1	SPECIAL	NO. 2 AND NO. 3
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
< 5.0	8	62.50	25.00	12.50
5.00–5.04	133	65.41	24.81	9.77
5.05–5.09	235	63.40	25.11	11.06
5.10–5.14	135	58.52	31.85	9.63
5.15–5.19	100	55.00	31.00	14.00
5.20–5.29	79	46.84	27.85	25.31
5.30–5.39	61	18.03	24.59	57.38
5.40–5.49	42	21.43	21.43	57.14
5.50 and over	12	25.00	25.00	50.00
All 5.2 and over	194	30.93	25.26	43.81
All < 5.2	623	61.32	27.45	11.23

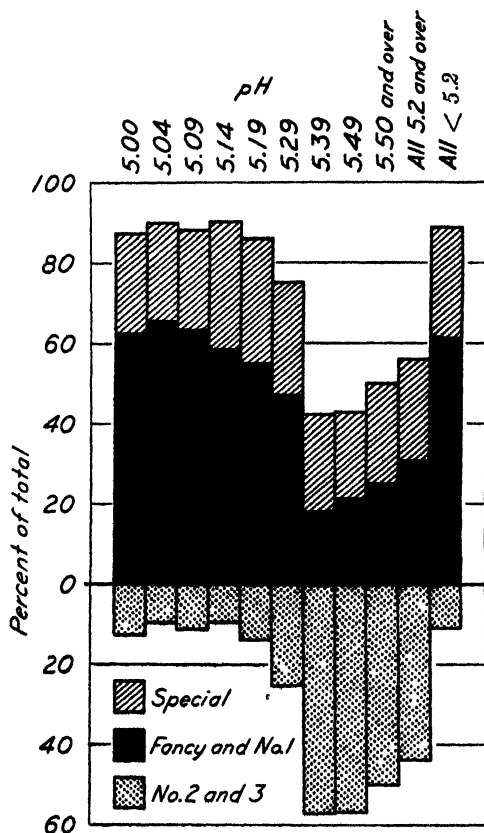


Fig. 1. Relation of pH at 21 hours to grade of cheese.

All of these cheeses showed a slow increase in acidity during the early hours in the press, but the increase was steady and undoubtedly continued after the 21-hour period. These results agree with the belief of experienced cheesemakers that the less starter it is found necessary to use, the more likely are good cheeses to result. The good results obtained with 39a

TABLE 7  
Changes in pH of all cheeses of good grade with 21-hour pH of over 5.2

Grade	3 HOURS		8 HOURS		10 HOURS		21 HOURS	
	No. of cheeses	Average pH	No. of cheeses	Average pH	No. of cheeses	Average pH	No. of cheeses	Average pH
Fancy	5	6.21			4	5.53	5	5.28
Good No. 1	20	6.10	12	5.85	3	5.66	25	5.32
Fair No. 1	25	6.08	16	5.79	4	5.64	50	5.35

starters with less than 0.9 per cent acidity in certain cases, as discussed above, were in cheeses in which there was slow development of acidity during the first hours in the press. In the presence of active gas-forming bacteria, however, slow working *Str. thermophilus* or *L. helveticus* starters or small amounts of them could not be used successfully.

#### DISCUSSION

Since it is usually possible to control to a great extent the rate of the lactic fermentation in Swiss cheese in the press by controlling the starters used in making the cheese, the cheesemaker should know first how to prepare the starters which will be most active in the cheese and, second, how much of these active starters should be used to cause the desired rate of lactic fermentation in the cheese in the press. The results of the above data would indicate that a 39a starter should have an acidity of about 1.05 to 1.09 per cent and the C<sub>3</sub> whey starter an acidity of 0.3 to 0.33 per cent. Enough C<sub>3</sub> starter should be added to cause the pH of the cheese to drop to about 6.0 to 6.09 in the first three hours of the cheese in the press and enough 39a starter should be added to give a pH of less than 5.2, preferably less than 5.15, after the cheese has been in the press for 21 hours. No changes can be made to influence the cheese on which the tests are run but the changes can be effected the next day. In practice it is not always possible to obtain the ideal pH value in the cheese, especially if the milk is defective. Some sources of milks are so infected with gas organisms that a large amount of *Str. thermophilus* starter is necessary to prevent bloating of the cheese in the press. The resulting cheese will probably not be of good quality but the "pressler" will have been avoided. Some milks are inhibitive to the lactobacillus starter and difficulty is experienced in obtaining a low enough pH value in the 21-hour old cheese. This trouble was encountered at the Grove City Creamery and it was found necessary to use a 39a starter grown at 38–39° C. with an acidity of about 1.1 to 1.15 per cent. And, with an exceptionally good milk, as has been stated above, a slow acting *Str. thermophilus* as well as a slow acting lactobacillus can be used with good results.

Of course the first requisite for the manufacture of Swiss cheese of high quality is satisfactory milk. And with good milk, correct manufacturing procedures and properly prepared and used starters, it should be possible to make Swiss cheese of high average quality.

#### SUMMARY

A study of a large number of wheels of Swiss cheese manufactured at a plant under factory conditions, in an effort to determine what acidity of pure culture starters and what rate of lactic acid fermentation in the cheese in the press were most desirable, led to the following conclusions:

1. With the milk used, a *Lactobacillus helveticus* milk starter with an acidity of 1.0 to 1.09 was most effective. This culture was grown at 37.5–39° C. for 12 hours.

2. A 12-hour, 37° C. milk starter of *Str. thermophilus* should have an acidity of about 0.70 to 0.75 per cent; and a whey starter should have an acidity of 0.30 to 0.33 per cent.

3. The pH of the cheese in the press, which is indicative of the activity of the starters, should be about 6.0 to 6.09 after three hours in cheese made from milk with a pH of 6.5–6.6.

4. The pH of the cheese in the press should be less than 5.2 after 21 hours and preferably should be between 5.15 and 5.0.

5. With good milk a slow development of acidity throughout the first 21 hours usually makes a good cheese. The pH of such cheeses may be as high as 6.1 to 6.25 after three hours in the press, and over 5.2 after 21 hours.

6. With a supply of good milk and with good manufacturing methods, the proper preparation and use of pure culture starters will give Swiss cheese of good average quality.

# VITAMIN D STUDIES IN CATTLE

## I. THE ANTIRACHITIC VALUE OF HAY IN THE RATION OF DAIRY CATTLE\*

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WITH THE TECHNICAL ASSISTANCE OF C. C. LIGHTFOOT

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For the past 12 years the calcium and phosphorus requirements of dairy cattle have been under investigation at this Station. During this period several long-time mineral feeding trials and many balance experiments have been carried out (1-4). In order to complete certain phases of this investigation, it became necessary to study the vitamin D requirement of dairy cattle and also the antirachitic value of common dairy feeds. These factors are essential for the proper utilization of calcium and phosphorus.

Extensive reviews of the literature bearing on the etiology of rickets and the effects of various curative agents have been made by Park (5), Hess (6), Blunt and Cowan (7), Sherman and Smith (8), and Browning (9). Consequently, only the literature pertaining directly to rickets in cattle will be given.

Rickets have been observed in calves by several early writers. In 1901 Law (10) listed it as a disease affecting cattle. In their 1914 edition, Huttyra and Marek (11) have a splendid photograph of a rachitic calf. In 1920 Becker (12) observed rickets in calves fed whole milk and grain. The California Station (13) reported that rachitic calves were common in the range herds of King County as early as 1922.

Rachitic calves were produced experimentally at this Station (14) as early as 1926 in connection with the heavy feeding of concentrates without the proper quality of roughage. Bechdel and coworkers (15) presented data in 1928 which indicated that vitamin D was beneficial to the bovine. Olson (29) reported in 1929 that rickets occurred among calves allowed free choice of feeds. The rachitic symptoms were associated with low hay intakes. In 1930 Huffman and coworkers (16) reported a case of rickets in a young animal which was fed a ration containing a limited amount of wheat straw. Hill (17) reported in 1930 that calves fed a rickets-producing ration and exposed to ultraviolet rays showed improved calcification of the bones. Rupel, Bohstedt and Hart (18) demonstrated in 1931 that rachitic

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calves responded promptly to the curative treatment of cod liver oil or sunshine. The effects of supplementing a rachitogenic ration with cod liver oil, sun-cured hay or sunshine were reported in 1931 (19). In 1932 Gullickson and Eckles (20) observed rickets in calves under farm conditions. In 1933 both the Pennsylvania Station (21) and the Wisconsin Station (22) reported in detail upon the vitamin D studies at their respective institutions. The characteristic symptoms of rickets in calves were described in the last two reports.

Hess and Weinstock (23) reported that green plants grown in the dark were devoid of antirachitic properties but such plants were rendered antirachitic by irradiation with ultraviolet rays. According to Steenbock and coworkers (24) the antirachitic potency of hay can also be increased by exposure to sunshine or to ultraviolet irradiation. Similar results have been reported by other investigators (25-27).

The purpose of this investigation is to help elucidate the vitamin D requirements of calves and also to study the antirachitic potency of hay.

#### EXPERIMENTAL

Twelve calves were used in the study of the antirachitic properties of hay. The calves were all healthy grade Holsteins except C-252 which was a Brown Swiss. From birth to 30 days of age the calves were fed whole milk twice daily in amounts according to the needs of each calf, after which time they were changed to a ration of skim milk and a mixture of corn and oats. At 90 days of age the corn and oats were replaced by the following basal rachitic grain mixture:

Yellow corn meal	50.0 per cent	Linseed oil meal	8.5 per cent
Ground oats	20.0 " "	Calcium carbonate	1.0 " "
Corn gluten meal	20.0 " "	Sodium chloride	0.5 " "

Monthly analyses of rachitogenic ration were made and showed the following variations:

Calcium	0.412 to 0.592	0.500 average per cent
Phosphorus	0.301 to 0.376	0.351 " " "
Magnesium	0.164 to 0.216	0.195 " " "
Nitrogen	2.92 to 3.41	3.10 " " "
Moisture	10.27 to 12.95	11.27 " " "

At 120 days of age the skim milk was discontinued. Five calves, C-98, C-100, C-109, C-148 and C-131 received sun-cured hay as an antirachitic supplement. Four calves, C-127, C-130, C-133, and C-165 received timothy hay which had been cured in the dark as a supplement. Two calves, C-216 and C-252, were fed sun-cured alfalfa hay as an antirachitic supplement. Calf C-144 was fed timothy ash as a supplement.

The sun-cured timothy hay was U. S. grade No. 2. The timothy which was cured in the dark was cut after sundown and placed upon racks in a dark room to cure. This hay was classed as U. S. grade No. 2. The sun-cured alfalfa which was U. S. Grade No. 1 was purchased from farmers within the vicinity of the Station. The vitamin D content of these hays was not determined by rat assays.

The calves were turned into a bare lot at night for exercise except during inclement weather. They were offered water twice daily. Shavings were used for bedding.

The animals were weighed every 10 days and measured for height at withers once a month.

Blood samples were obtained every 2 weeks, in many cases each week, from all of the experimental animals from the time they were placed on experiment until they were removed from experiment. The methods for the determination of plasma calcium and inorganic phosphorus have been previously reported (3).

The right 8th rib and in a few cases the right dental pad and a section of the orbital plate of each calf were cleaned, air-dried, sawed into desirable lengths, extracted with hot alcohol for 72 hours and finally dried in an oven. The samples were weighed into crucibles and ashed in a muffle for 12 to 15 hours. After cooling in a desiccator they were reweighed. The ashed samples were ground to pass a 20 mesh sieve. Aliquot samples were then taken for the determination of calcium, phosphorus and magnesium.

The methods for the collection of the excreta and of the analysis were the same as in previous work (28).

#### RESULTS

The results for representative calves are presented in Tables 1-7 inclusive.

*Calf C-144.* Timothy ash equivalent to 2 pounds of timothy hay per day was added to the ration of C-144 at 90 days of age to compensate for the calcium, phosphorus and magnesium contained in the hay. The plasma calcium and inorganic phosphorus had been normal up to this time. By 105 days of age the inorganic phosphorus had dropped to 5.61 mg. per 100 cc. of plasma. The symptoms of rickets were marked at 145 days of age although the plasma inorganic phosphorus had been low for some time. Table 1 shows a typical case of low blood inorganic phosphorus rickets. Several calves not included in this discussion, which had received only the basal rachitic ration, also became extremely rachitic.

#### *Effect of Supplementing the Basal Ration with Sun-cured Timothy Hay*

In order to determine the antirachitic value of sun-cured hay, 5 calves were fed the basal rachitic ration supplemented with sun-cured timothy.

TABLE 1  
C-144. Effect of supplementing the rachitogenic ration with timothy ash

DATE	AGE	WT.	PLASMA		DATE	AGE	WT.	PLASMA	
			Ca	P				Ca	P
	<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>			<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>	
10-12-31	40	94	12.6	7.49	12-31-31	120	159	10.5	5.51
10-22	50	100	11.3	6.25	1-10-32	130	168		
11-1	60	111			1-20	140	182	10.9	3.95
11-11	70	115	11.0	8.33	1-30 <sup>2</sup>	150	169	10.2	4.46
11-21	80	119	10.2	8.01	2-9	160	185		
12-1 <sup>1</sup>	90	124			2-19	170	187	10.4	4.43
12-11	100	131	11.2	5.61	2-29	180	194		5.53
12-21	110	140			3-10	190	200	9.6	4.11

<sup>1</sup> Timothy ash added.

<sup>2</sup> Legs bowed.

*Calf C-98.* This calf received alfalfa hay and 10 cc. of cod liver oil per day to 90 days and was then changed to the basal ration. The calcium and inorganic phosphorus values were 12.0 and 5.25 mg. per 100 cc. of plasma respectively at 150 days of age, as indicated in Table 2. The phosphorus value was slightly below the normal range for a calf of that age. At 193 days of age the plasma calcium and inorganic phosphorus values were 7.0 and 6.95 mg. respectively and by 210 days of age anorexia was exhibited. Stiffness was not observed until about 225 days of age. This animal suffered from low plasma calcium, normal phosphorus rickets at this time. It is of interest to note that tetany was not observed although the plasma calcium values were below 6.0 mg.

Sun-cured timothy hay was added to the ration at 254 days of age when the calf was suffering severely with rickets. The plasma calcium immediately increased to 11.3 mg. but the inorganic phosphorus remained unchanged. It was planned to feed 3 pounds of timothy per day but due to anorexia, the calf would not consume this amount of hay until about 30 days from the time it was added to the ration.

During the period that rickets had developed the calcium and phosphorus intakes were approximately 14.5 and 9.1 gm. per day. After the sun-cured timothy had alleviated the anorexic and rachitic conditions the above intakes were increased to 20.0 and 11.3 gm. respectively. The addition of the hay also changed the calcium to phosphorus ratio from 1.6:1 to 1.8:1.

After the sun-cured timothy had been added to the ration, all of the plasma calcium values and most of the inorganic phosphorus values were normal until this animal was 540 days of age, but at this age the inorganic phosphorus values began to decrease. C-98 started to bloat at 543 days of age and anorexia was again manifested. The grain consumption began

TABLE 2  
C-98. Effect of supplementing the rachitogenic ration with sun-cured timothy hay

DATE	AGE	WT.	PLASMA		DATE	AGE	WT.	PLASMA	
			Ca	P				Ca	P
		lbs.	mg. per 100 cc.			days	lbs.	mg. per 100 cc.	
12-19-30	150	276	12.0	5.25	5-12	660	636	13.4	5.39
1-19-31	180	310			6-11	690	640	14.2	5.49
2-17	210	305	6.5	8.65	7-11	720	708	14.5	4.92
3-19 <sup>1</sup>	240	299	5.8	7.47	8-10	750	757	13.7	5.91
4-22	254	301	7.0	5.95	9-9	780	796	12.6	5.40
4-18	270	297	11.3	5.87	10-9 <sup>4</sup>	810	839	12.3	5.32
5-18	300	325	11.3	6.14	11-8	840	887	11.3	5.90
6-17	330	351	11.9	6.76	12-8-32	870	926	11.9	6.23
7-17	360	391	11.5	5.82	1-7-33	900	947	12.0	6.80
8-16	390	432	12.4	6.24	2-6	930	997	10.8	6.33
9-15	420	465	11.4	7.13	3-8	960	1054	11.0	5.52
10-15	450	487	11.7	6.57	4-7	990	1087	12.1	5.71
11-14	480	536	11.3	5.95	5-7	1020	1120	11.3	6.06
12-14	510	573	11.3	6.21	6-6	1050	1147	11.0	5.59
1-13-32	540	574	11.4	5.89	6-30 <sup>5</sup>	1074	967	11.9	3.71
2-12	570	590	10.8	5.16	7-6	1080	973	10.0	4.19
2-29 <sup>3</sup>	587	588		6.04	8-5	1110	990	9.8	4.24
3-13	600	576		6.04	9-5	1140		11.2	5.38
4-12	630	608	12.5	5.18	9-29	1164	990	11.9	6.62

<sup>1</sup> Marked rickets.<sup>2</sup> 3 lbs. sun-cured hay added per day.<sup>3</sup> Hay increased to 4 lbs. per day.<sup>4</sup> Conceived.<sup>5</sup> Ca<sup>2</sup>ed.

to decrease but the 3 pounds of hay were readily consumed. Stiffness was observed at 585 days of age or 42 days following the initial bloating and development of anorexia. The plasma inorganic phosphorus was slightly below normal during this period but the calcium was normal. The hay intake was increased at 587 days of age but it required about 30 days to get it up to 6 pounds per day. The appetite for grain increased at a slower rate as four months were required to get the consumption up to the level just prior to the last siege of anorexia and attack of rickets. As the appetite for grain improved, the appetite for timothy decreased, which, together with good gains in body weight, were probably responsible for the inorganic phosphorus values remaining slightly below normal.

C-98 manifested estrum and was bred at 555 days of age but conception did not occur. She did conceive at the second service at 814 days of age. The calf, which was carried 261 days and was dead at birth, weighed 82 pounds. Post-mortem examination did not reveal any abnormalities. All of the bones of the calf were normal and well calcified. The placenta was discharged within the normal time. This animal had suffered from rickets on two different occasions yet she did not experience difficulty in calving. She was kept for 88 days following parturition.

The data for the above period showing milk production, calcium, phosphorus and magnesium intakes, the grams of food phosphorus per pound of milk above maintenance, plasma calcium and inorganic phosphorus appear below:

PERIOD	WEIGHT	HAY	MILK	AVER. DAILY INTAKE			P INTAKE <sup>2</sup>	PLASMA	
				Ca	P	Mg		Ca	P
<i>days</i>	<i>lbs.<sup>1</sup></i>	<i>lbs.</i>	<i>lbs.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>mg. per 100 cc.</i>	
31	967	6	23.4	35.3	20.1	13.8	0.446	10.9	3.95
31	990	6	30.8	52.5	26.3	20.3	0.532	9.8	4.24
26	990 <sup>3</sup>	6	27.6	66.5	35.0	25.8	0.909	11.5	6.00

<sup>1</sup> Weight at beginning of period.

<sup>2</sup> Per pound of milk above maintenance. Maintenance requirement is 1 gram of phosphorus per 100 pounds of body weight.

<sup>3</sup> Weight at end of last period.

During the first and second months when the phosphorus intakes per pound of milk above maintenance were 0.446 and 0.532 gm., the plasma inorganic phosphorus values were low for a heifer of this age. During the third month the consumption of grain had increased to 15 pounds per day and consequently the phosphorus intake increased to 0.909 gm. per pound of milk above maintenance which caused the plasma inorganic phosphorus to return to normal. There was a pronounced improvement in the general

condition of C-98 during the time she was milking. The stiffened and enlarged joints, which were so pronounced at the time of calving, had almost disappeared by the time she was slaughtered.

It is of importance to call attention to the fact that 6 pounds of sun-cured timothy hay was the only source of vitamin D and that this amount of hay provided sufficient vitamin D for maintenance, some growth and the production of 23.4 to 30.8 pounds of milk per day. Apparently, the improved calcium, phosphorus and magnesium intakes were important factors in helping to restore the plasma inorganic phosphorus to normal. The metabolism data for calcium, phosphorus, magnesium and nitrogen following the recovery from the first attack of rickets are shown in Table 3. The

TABLE 3  
*Metabolism data of C-98 and C-100 fed the rachitogenic ration supplemented with sun-cured timothy hay*

CALF NO.	AVER. DAILY INTAKE	AVER. DAILY OUTGO			BALANCE	% STORED	PLASMA	
		Urine	Feces	Total			Ca	P
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		<i>mg. per 100 cc.</i>	
C-98 <sup>1</sup> Ca	21.77	0.36	10.40	10.76	11.01	50.6	11.3	6.56
P	12.95	3.46	3.88	7.34	5.61	43.3		
Mg	7.87	2.00	2.99	4.99	2.88	36.6		
N	94.73	49.17	21.75	70.92	23.81	25.1		
C-100 <sup>2</sup> Ca	19.45	0.02	12.28	12.30	7.15	36.8	11.2	6.65
P	12.04	2.94	5.38	8.32	3.72	30.9		
Mg	7.15	1.94	2.83	4.77	2.38	33.2		
N	90.46	54.02	24.09	78.11	12.35	13.6		

<sup>1</sup> 3 lbs. of hay per day. Age 363 days. Weight 417 lbs.

<sup>2</sup> 2 lbs. of hay per day. Age 296 days. Weight 410 lbs.

metabolism period lasted one week but no feed changes were made for several weeks prior to the collection period. The ash values for the moisture-free, fat-free 8th right ribs of C-98 and her calf are shown in Table 7.

*Calf C-100.* This calf consumed 15.4 pounds of sun-cured alfalfa hay in addition to the basal ration during the first 60 days of age. At this time the alfalfa was replaced by 2 pounds of sun-cured timothy hay per day. The body weights, plasma calcium and inorganic phosphorus values are shown in Table 4.

Two pounds of sun-cured timothy hay furnished a sufficient amount of the antirachitic factor to maintain a normal concentration of calcium and inorganic phosphorus in the plasma to about one year of age. The inorganic phosphorus values were slightly subnormal after this time but anorexia was not observed until 504 days of age and stiffness was observed 4 days later. The phosphorus intake during this period ranged from 10.6 to 15.2 gm. per day. The hay was increased to 4 pounds per day on the 519th day. Slight improvement in the rachitic condition was first observed

TABLE 4  
Effect of supplementing the rachitogenic ration with sun-cured timothy hay

DATE	AGE days	WT. lbs.	PLASMA		DATE	AGE days	WT. lbs.	PLASMA	
			Ca	P				Ca	P
			mg. per 100 cc.					mg. per 100 cc.	
Calif C-100									
1-25-31	120	223	11.2	6.54	3-20-32	540	589	11.5	4.18
2-23	150	243	11.2	7.81	4-19	570	620	12.4	5.08
3-26	180	257	10.7	8.20	5-18	600	618	12.1	5.27
4-25	210	293	10.6	7.82	6-18	630	670	13.7	6.35
5-25	240	327	10.5	7.04	7-18	660	676	14.2	6.04
6-24	270	376	10.7	6.70	8-17 <sup>2</sup>	690	732	13.7	5.67
7-24	300	415	10.8	6.53	9-16	720	740	12.7	5.53
8-23	330	431	11.0	5.77	10-16	750	745	11.4	5.46
9-22	360	469	10.8	6.65	11-15	780	754	10.8	5.62
10-22	390	497	11.9	6.12	12-15-32	810	765	11.6	5.49
11-21	420	506	10.8	5.60	1-14-33	840	779	11.8	6.06
12-21-31	450	533	11.1	7.09	2-13	870	808	11.5	6.63
1-20-32	480	543	11.3	6.41	3-15	900	855	11.4	6.29
2-19 <sup>1</sup>	510	575	10.6	4.75	4-7 <sup>3</sup>	923	846	13.0	5.81
Calif C-148									
3-21-32	20	98	12.5	5.61	7-19	140	165		
3-31 <sup>4</sup>	30	110	11.9	4.46	7-29 <sup>7</sup>	150	165	12.0	3.12
4-10	40	118	11.3	5.84	8-8	160	167	10.4	2.58
4-20	50	129	11.4	6.22	8-18	170	172	9.7	3.93
4-30	60	144	10.1	5.43	8-28	180	170		
5-10	70	154			9-7	190	171	9.9	2.96
5-20	80	166	10.0	5.04	9-17	200	175	9.6	3.23
5-30 <sup>5</sup>	90	171	11.7	5.32	9-27	210	180		
6-9	100	171	11.8	4.09	10-7	220	190	12.3	5.00
6-19	110	169			10-17	230	187	8.5	4.05
6-29 <sup>6</sup>	120	168	12.2	3.95	10-19-32	232		7.3	4.13
7-9	130	165	11.8	2.76					

<sup>1</sup> Anorexia and stiffness, sun-cured timothy in-

creased to 4 lbs. per day.

<sup>2</sup> Hay increased to 6 lbs. per day.

<sup>3</sup> Slaughtered.

<sup>4</sup> Sun-cured timothy hay added to ration.

<sup>5</sup> Rickets.

<sup>6</sup> Added MgO to ration.

<sup>7</sup> Added bone-meal to ration.

at 543 days of age but this condition was probably due to lack of growth. The plasma calcium was normal but the inorganic phosphorus was subnormal. The improved condition only lasted about 30 days when the stiffness became more pronounced. At 681 days of age the timothy was increased to 6 pounds per day. There was another period of gradual improvement in the rachitic condition but anorexia was the predominant factor. The calcium and phosphorus intakes at this time were 25 and 15 gm. respectively. By 850 days of age anorexia had disappeared and the grain consumption had increased to such an extent that C-100 gained in weight and in height at withers and at 909 days she weighed 861 pounds. The stiffened and enlarged joints had almost disappeared but the inorganic phosphorus values remained below 6.5 mg. in most cases. C-100 was slaughtered at 923 days of age because of a foreign body. She had had 13 services but conception had not occurred.

The metabolism values for calcium, phosphorus, magnesium and nitrogen just prior to the development of rickets are shown in Table 3. The collection period lasted for one week but no feed changes were made for many weeks previous to this time. The ash values are shown in Table 7.

*Calf C-109.* Blood samples were not taken from this calf until 84 days of age but the calcium and inorganic phosphorus values at that time were 7.6 and 7.40 mg. per 100 cc. of plasma. One pound of sun-cured timothy hay was added to the basal rachitic ration at 87 days of age. The plasma calcium values remained below the normal range until the calf was slaughtered at 188 days of age. The inorganic phosphorus values were normal until 160 days of age but from then on they were below normal. Tetany was not observed at any time and the calf did not appear irritable. The knee joints began to enlarge but stiffness had not been observed. Post-mortem examination revealed the usual rickety bones.

*Calf C-148.* Table 4 summarizes the data secured from C-148. This calf had low inorganic phosphorus values when it was bled at 20 and 30 days of age but the calcium values were normal. Sun-cured timothy hay was added to the ration at 30 days of age. The calf was offered more but would not consume more than one pound per day up to 90 days of age. Evidence of rickets was observed at this time when the calcium was 11.7 and the inorganic phosphorus was 5.32 mg. per 100 cc. of plasma. The rachitic condition became progressively worse and at 121 days of age, 10 gm. of magnesium oxide were added to the ration in an attempt to relieve this condition. There was an immediate increase in the consumption of hay and grain but the inorganic phosphorus continued to decrease. The body weight remained constant and the rachitic symptoms remained unchanged even after the hay and grain intakes were doubled. Fifty grams of bone-meal per day were added to the ration at 153 days of age but the increased calcium and phosphorus intakes did not prevent the calf from becoming

extremely rachitic. The condition was such that the animal could not walk without assistance. All other feeds were replaced with 20 pounds of whole milk at 211 days of age. The plasma calcium, inorganic phosphorus and magnesium values at 220 days of age were 12.3, 5.00 and 2.12 mg. per 100 cc., respectively. At 232 days of age this calf had tetanic convulsions, during which time the calcium, inorganic phosphorus and magnesium values were 7.3, 4.13 and 0.99 mg., respectively. This calf presented a typical case of low-calcium, low-phosphorus rickets. The cause of the tetany may have been due either to the low concentration of calcium in the plasma, the low concentration of magnesium, or the subnormal concentrations of both of these elements.

*Calf C-131.* The concentrations of calcium and inorganic phosphorus in the plasma of this calf were normal during the first 46 days of age. At 65 days of age the plasma calcium dropped to 7.6 mg. per 100 cc. but the inorganic phosphorus remained within the normal range. Sun-cured timothy hay was added to the ration at 93 days of age. This was sufficient to maintain the normal concentration of inorganic phosphorus until 136 days of age. The first evidence of rickets was observed at 178 days when both the plasma calcium and inorganic phosphorus were much below normal.

Considerable difficulty was encountered in getting this calf to consume enough nutrients either as hay or grain. Originally it had been planned to feed the calf 2 pounds of sun-cured timothy but due to anorexia, the amount of hay consumed was usually about 1 pound per day.

At 239 days of age C-131 became extremely rachitic and was only able to get up with considerable difficulty. The calf was changed to another experiment at this time.

#### *Effect of Feeding Timothy Hay Cured in the Dark*

*Calf C-127.* The first indications of rickets were observed in this calf at 84 days of age when the calcium was 7.8 mg. per 100 cc. of plasma but the inorganic phosphorus was normal. One week later tetany was manifested so the ration was supplemented with timothy hay which had been cured in the dark. The plasma calcium and inorganic phosphorus values at this time were 6.4 and 7.53 mg., respectively. Tetany was observed at 94 and 95 days of age. Difficulty was encountered in getting this animal to consume hay and at no time did it consume more than 1.5 pounds per day. By 165 days of age the hay consumption had decreased to less than 1 pound per day. It was impossible to determine the antirachitic value of this hay because of the small amount consumed. However, the feeding of sun-cured timothy from the same field and at the same level was also ineffective in curing or preventing rickets (Calf C-109). The plasma calcium and inor-

ganic phosphorus values were all markedly subnormal during the last 3 months of life.

*Calf C-130.* The plasma calcium and inorganic phosphorus values were normal until 76 days of age at which time the calcium was 8.6 mg. per 100 cc. The inorganic phosphorus remained normal until 133 days of age. Timothy hay which had been cured in the dark was added at 99 days of age. Difficulty was encountered in getting C-130 to consume hay although about 2 pounds per day were consumed for 2 months. This amount of hay would not have been sufficient to cure rickets even though it had been sun-cured. The calf was removed from this phase of the experiment at 269 days of age.

*Calf C-133.* The plasma calcium and inorganic phosphorus of this calf was also normal until 73 days of age when the calcium dropped to 9.2 mg. per 100 cc. The inorganic phosphorus remained within the normal range until 126 days of age. Timothy hay which had been cured in the dark was added to the ration at 92 days of age but the consumption did not exceed 1.5 pounds per day at any time. The calf was slaughtered at 170 days of age at which time the plasma calcium and inorganic phosphorus values were 8.5 and 4.51 mg. per 100 cc.

*Calf C-165.* The timothy hay which was cured in the dark was added to the ration of this calf at 40 days of age while the plasma calcium and inorganic phosphorus values were still normal but the calf would not eat enough hay to prevent the onset of rickets. The calf began to manifest anorexia even though the plasma calcium and inorganic phosphorus values were still normal. The average daily consumption during the 152 days of hay feeding was only 0.5 pound. It is of interest to note that the blood plasma indicated the simultaneous development of the low-calcium, low-phosphorus type of rickets, as shown in Table 5.

TABLE 5  
*C-165. Effect of supplementing the rachitogenic ration with timothy hay cured in the dark*

DATE	AGE	WT.	PLASMA		DATE	AGE	WT.	PLASMA	
			Ca	P				Ca	P
	<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>			<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>	
5-5-32	20	101	13.1	6.44	8-13	120	191		
5-15	30	110	12.4	6.51	8-23	130	194	8.7	4.30
5-25 <sup>1</sup>	40	123	11.9	6.28	9-2	140	189	8.9	4.63
6-4	50	134			9-12	150	192		
6-14	60	145	10.7	7.35	9-22	160	196	8.5	4.41
6-24	70	150	10.1	7.81	10-2	170	199	10.5	4.96
7-4	80	158			10-12	180	196	6.6	3.62
7-14	90	164	10.7	8.68	10-22	190	201		
7-24	100	170	9.0	5.93	10-24-32	192	203	7.7	3.33
8-3	110	186	8.9	5.63					

*Antirachitic Effect of Sun-Cured Alfalfa Hay*

*Calf 216.* This calf received the basal rachitic ration supplemented with sun-cured alfalfa. The results are given in Table 6. In view of the

TABLE 6  
*C-216. Effect of supplementing the rachitogenic ration with 2 pounds of sun-cured alfalfa hay*

DATE	AGE	WT.	PLASMA		DATE	AGE	WT.	PLASMA	
			Ca	P				Ca	P
	<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>			<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>	
9-25-33	30	128	10.9	7.49	12-24-33	120	320	12.5	6.65
10-5	40	146	11.6	7.44	1-3-34	130	327	12.6	5.84
10-15	50	170	11.8	8.06	1-13	140	338	12.7	6.25
10-25	60	190	11.3	7.44	1-23	150	350	12.9	6.41
11-4	70	211	12.1	8.43	2-2	160	360	12.4	7.01
11-14	80	234	11.8	8.23	2-12	170	370	11.4	5.41
11-24	90	259	13.0	9.03	2-22	180	388	11.7	7.01
12-4	100	285	13.1	7.35	3-4	190	410	12.2	8.12
12-14	110	306	12.6	7.34	3-9 <sup>1</sup>	195	421	12.5	7.27

<sup>1</sup> Slaughtered.

TABLE 7  
*Ash and mineral analyses of bones of calves fed the rachitogenic ration with various supplements*

8TH R. RIB					ORBITAL PLATE				DENTAL PAD			
<i>Calf No.</i>	<i>Ash %</i>	<i>Ca %</i>	<i>P %</i>	<i>Mg %</i>	<i>Ash %</i>	<i>Ca %</i>	<i>P %</i>	<i>Mg %</i>	<i>Ash %</i>	<i>Ca %</i>	<i>P %</i>	<i>Mg %</i>
					Timothy Ash							
C-144	41.46	16.58	7.23	0.53	43.25				46.69			
					Timothy—Sun Cured							
C-98	57.50	21.45	9.67	0.70								
C-98's	53.00	20.80	9.52	0.69								
C-100	60.65	23.05	10.30	0.38	59.25	23.00	10.88	0.88	54.10	20.76	10.18	0.84
C-109					49.70	18.98	8.87	0.81	47.42	18.49	8.53	0.51
C-148	42.89	17.65	7.69	0.57	36.81	14.58	6.62	0.68	58.44	18.14	7.78	0.89
					Timothy—Cured in Dark							
C-127	42.50	17.33	7.62	0.55	39.88				42.42			
C-133	47.68	18.98	8.06	0.51	46.07				48.74			
C-165	41.48	16.75	7.35	0.48	33.85	13.34	6.08	0.65	54.92	17.85	7.78	0.89
					Alfalfa—Sun Cured							
C-216	52.30	19.87	8.93	0.58	56.30							
C-252	55.10	21.35	10.28	1.01	21.40 10.11 0.96							

results secured from the feeding of timothy hay, the alfalfa was added at 8 days of age and limited to not more than 2 pounds per day in order to overcome anorexia. The plasma calcium and inorganic phosphorus values were normal throughout the duration of the experiment. No evidence of rickets was observed. The high calcium content of the alfalfa altered the

calcium to phosphorus ratio of the rachitogenic ration from 1.6:1 to 2:1 and 3:1. The high magnesium content of the alfalfa may also have aided in the prevention of rickets. The growth and condition of the calf at the time of slaughter was normal.

*Calf C-252.* The ration of this calf was supplemented with 1 pound of sun-cured alfalfa hay per day at 30 days of age. This amount of hay furnished enough of the antirachitic factor to maintain normal plasma calcium and inorganic phosphorus values up to 130 days of age. The skim milk was discontinued at 120 days of age which caused a temporary drop in the calcium and phosphorus intakes. The inorganic phosphorus dropped to 5.71 mg. per 100 cc. of plasma but as soon as the consumption of grain had increased sufficiently to meet the calcium and phosphorus requirements the plasma phosphorus quickly returned to normal. This indicates the importance of the level of calcium and phosphorus in the ration in studying the vitamin D requirements of young dairy animals.

#### DISCUSSION

Anorexia was one of the first symptoms observed in the calves used in this investigation to determine the antirachitic value of hay. Several calves lost their appetite for the basal rachitic grain mixture as well as for hay. Calf C-100 had consumed 15.4 pounds of sun-cured alfalfa hay from birth to 60 days of age and did not exhibit anorexia during her first year when 2 pounds of sun-cured timothy hay were the only source of the antirachitic factor. It is possible that anorexia was prevented in C-100 by feeding the sun-cured hay at an earlier age. On the other hand, C-98 had been raised to 90 days of age on a ration of sun-cured alfalfa and 10 cc. of cod liver oil per day in addition to the basal ration. This procedure did not prevent the occurrence of anorexia in this calf during the period of active rickets. C-148 was offered sun-cured timothy hay at 30 days of age but it refused to consume more than 1 pound per day which was ineffective in the prevention of anorexia. Most of the calves which were used in this investigation failed to consume the desired amount of hay.

Calf C-98 was cured of rickets with sun-cured timothy hay. The calf was limited to 3 pounds per day for some time but the marked improvement in the rachitic condition, as the result of the consumption of sun-cured hay, demonstrated the antirachitic potency of such a hay. Although this amount of sun-cured hay alleviated the rachitic symptoms in C-98 at 9 months of age, it failed to furnish enough vitamin D to prevent the reoccurrence of rickets at 15 months of age. After the second onset of rickets the hay was gradually increased to 6 pounds per day. This amount of hay furnished sufficient vitamin D to maintain the heifer, the development of the fetus and the average daily production of 27 pounds of milk. The results with calf C-100 indicate that 2 pounds of sun-cured timothy hay afforded ample protection against rickets up to about 1 year of age and that the gradual

increase in the hay consumption up to 6 pounds per day furnished sufficient vitamin D for maintenance and normal growth up to 2.5 years of age. Sun-cured alfalfa hay also had a marked antirachitic value as 2 pounds per day protected C-216 against rickets up to 195 days of age and 1 pound per day was effective in preventing rickets in C-252 up to 192 days of age. These results are in agreement with those reported by the Pennsylvania Station (21) where 2.5 pounds of sun-cured alfalfa protected calves from rickets while 1 pound prevented them from showing the clinical symptoms but failed to maintain normal blood inorganic phosphorus.

The calcium, phosphorus, magnesium and nitrogen balances in Table 3 demonstrate the antirachitic potency of sun-cured hay. Calf C-98 which received 3 pounds of hay per day as the only source of vitamin D had better utilization and storage of calcium and phosphorus than C-100 which received 2 pounds of hay per day.

After anorexia became pronounced the calves failed to make normal growth and in some cases growth ceased entirely or the animal lost weight. Some variations are shown, however, upon examination of the weights. The differences are probably due to individual variations in the ability of the calf to utilize the vitamin D supplied by the ration.

The results with the hay cured in the dark are of little value due to the manifestation of anorexia which prevented the consumption of sufficient hay to give it a fair trial.

Several types of rickets developed in the calves which were used in this investigation from the standpoint of blood analyses. The usual type was the low-plasma calcium, normal-inorganic phosphorus as indicated during the first attack of rickets of C-98. During the second attack, when sun-cured hay was fed, the condition was characterized by the normal calcium, low-inorganic phosphorus type. The latter type prevailed in the case of C-148, C-144 and C-100. The rachitic condition was characterized in C-133 and C-165 by the simultaneous drop in both plasma calcium and phosphorus. In cases where the first type of rickets prevailed, the inorganic phosphorus eventually became subnormal as the severity of the rickets increased.

The first alteration that appeared in the blood of the calves used in this investigation was a definite decrease in the concentration of calcium or inorganic phosphorus. When the Briggs modification of the Bell-Doisy technique is used, concentrations below 6.5 mg. per 100 cc. of plasma should be regarded as indicative of rickets, as the normal concentrations lie between 6.5 and 8.0 mg. for calves of the ages herein reported. It is quite probable that no rigid line can be drawn between the normal and pathological level of inorganic phosphorus because of individual variations and natural variations within the normal range. When the plasma calcium and inorganic phosphorus are determined at weekly intervals, the downward trend of either or both of these constituents preceded all other evidence.

The most significant features of the rachitic bones were their altered shapes, softer, less dense than normal, and their low ash and mineral content. The most outstanding observation, however, was the presence of pits in the articulating surfaces of the long bones of calves C-98 and C-100. A study of the pathology of rachitic calf bones will be reported in a subsequent paper.

#### SUMMARY AND CONCLUSIONS

1. Anorexia, which is usually associated with rickets in calves, was responsible for the lack of hay consumption under the conditions of this investigation.

2. It is difficult to use the curative method with calves when testing the antirachitic potency of roughages.

3. Two pounds of sun-cured timothy hay prevented rickets in calf C-100 up to 1 year of age and 3 pounds of sun-cured timothy cured rickets in C-98 at 9 months of age.

4. Two pounds of sun-cured alfalfa hay prevented rickets in calf C-216 up to 195 days of age and 1 pound of sun-cured hay protected C-252 up to 192 days of age.

5. The results indicate that the vitamin D requirement increases with age or size of the growing bovine and also that the requirement varies from individual to individual.

6. The calves used in this investigation showed three distinct conditions associated with rickets from the standpoint of blood analyses, (1) normal calcium, low phosphorus, (2) low calcium, normal phosphorus, and (3) low calcium, low phosphorus.

#### *Addenda:*

Since the acceptance of this paper for publishing G. C. Willis, L. S. Palmer and T. W. Gullickson reported that prairie hay may carry appreciable amounts of vitamin D.

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## PERIOD OF LACTATION AND THE DIRECT TITRATABLE CHLORIDE VALUE OF MILK

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### INTRODUCTION

The following types of natural milk may be high in chloride: (1) colostrum milk, (2) milk from diseased udders, and (3) milk from cows low in production, due to late lactation. In order to distinguish these three types from normal milk, a knowledge of the chloride content of the milk from healthy cows throughout the whole period of lactation is necessary.

The chloride test for detection of milk from diseased udders, particularly from cows having mastitis, has recently been used by Rosell (27), Hucker, Trudell and Jennings (9), Hayden (7), and others. Sharp and de Tomasi (30) have used the same test for a different purpose; namely, for the detection of milk from cows low in production, due to late lactation.

Eckles and Shaw (4) called attention to the fact that milk from cows late in lactation becomes bitter on standing. Palmer (16) (17) attributed this bitterness to the fatty acids liberated by the increased amount of lipase in the milk at the end of the lactation. Rogers (25), Rogers, Berg and Davis (26), Rice and Markley (21), Virtanen (37), Supplee (33), Nair (15), Dörner and Widmer (3), Sharp and de Tomasi (30), and others, have found that normal milk contains some lipase. Hammer and Bailey (6) and Koestler, Roadhouse and Lörtscher (12) called attention to the relation between bitter flavor, end of lactation, and milk high in chloride. The actual ratio of chloride to lactose was found to affect flavor by Gabathuler (5), Roadhouse and Koestler (24) and Roadhouse and Henderson (23).

In general, the more severely the milk secreting mechanism is injured by disease, or the longer a relatively small amount of milk is held in the udder, the more nearly the composition of the milk approaches that of the blood. This is evidenced by changes in the following milk constituents as shown by Storch (31) (32) Trunz (34), Eckles and Shaw (4), Koestler (10) (11) (13) and others: catalase, pH, titratable acidity, proteins, fat, lactose, pigments, sodium, potassium, calcium, phosphates and chloride: some exceptions occur. Colostrum milk has a relatively high titratable acidity, phosphate, and calcium content and a low pH. Both late lactation and mastitis milk show some of the same changes in composition and therefore respond alike to many tests for abnormality.

Many of the changes listed above have been suggested as a means of

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recognizing abnormal milk. Höyberg (8) found that the determination of the reaction (pH) of the milk colorimetrically with rosolic acid was a suitable method for recognizing the milk diseased udders. Baker and Van Slyke (2) studied the determination of the pH of abnormal milk colorimetrically and Baker and Breed (1) after comparing the use of rosolic acid, brom thymol blue, and brom cresol purple, recommended the latter. Brom thymol blue is now a more popular indicator, the use of which in detecting mastitis milk has been studied by Rosell (27), Udall and Johnson (35), Hucker, Trudell and Jennings (9), Plastring and Anderson (18), and others. This test is often positive (greenish-blue) for late lactation as well as for mastitis milk, and as recorded by Plastring and Anderson colostrum milk also changes this indicator to a greenish-blue color. The latter effect is due to an indicator error, since colostrum milk is notably low in pH. Gabathuler (5) suggested the use of the decrease in lactose, and Hammer and Bailey (6) the increase in chloride, determined by direct titration. Koestler (10) and his coworkers used what they called the chloride-lactose number which is 100 times the quotient obtained by dividing the amount of chloride by the amount of lactose

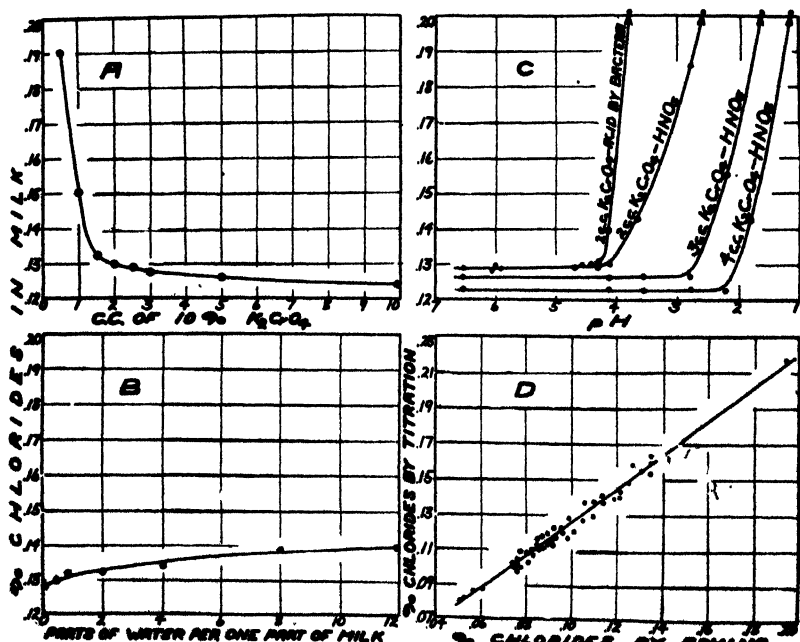


FIG. 1. Factors influencing the accuracy of the direct titration of milk for chloride. A, amount of indicator; B, dilution of the sample; C, acidity of the milk; D, amount of chloride as determined by ashing and by direct titration.

in the milk. The quotient changes proportionately more than either alone. Rosell (27) used changes in pH, chloride, catalase, and lactose to detect mastitis milk. Rullmann (28) and others found the leucocyte determination well adapted for the recognition of mastitis milk.

The direct titration of milk for chloride seems to be the simplest of the quantitative methods for the recognition of abnormal milk. Richmond (22) described the direct titration of milk with silver nitrate, using potassium chromate as an indicator, as a method for the detection of added salt. Hammer and Bailey (6) found that the values were uniformly about 0.026 per cent too high (Fig. 1, Part D), due to the effect of casein. Poetschke (19) (20) and Van der Burg and Koppenjan (36) criticized the direct titration method because of this error and the possible variation in it due to differences in protein. The data of Hammer and Bailey as plotted in Figure 1, Part D, show a definite linear relation, with only slight deviations, between the chloride determined by ashing and by direct titration of the milk. The variations due to the difference in protein would usually not be over  $\pm 0.005$  per cent of titratable chloride, except with colostrum milk. These considerations indicate that the direct titration of milk with silver nitrate would serve as a practical method for indicating any appreciable variation in chloride content. Rather than apply a correction factor, it is suggested that results obtained in this way be called *direct titratable chloride values*, bearing in mind that the true chloride content of the milk is less.

#### EXPERIMENTAL

##### *Method of titrating*

The procedure used in this paper was to transfer 18 grams of milk with a Babcock pipette to a 400 cc. beaker, add 2 cc. of a 10 per cent solution of potassium chromate, and titrate to a definite orange-red color with silver nitrate solution (8.624 grams per liter) of such a concentration that 1.00 cc. corresponded to 0.01 per cent of chloride in 18 grams of milk. Duplicate determinations usually fall within a range of  $\pm 0.20$  cc.

Some of the variations in titration procedure which influence the results were studied and are shown graphically in Figure 1. Part A shows that as the amount of indicator is increased the direct titratable chloride value rapidly decreases until about 1.5 cc. of potassium chromate has been added, and from then on an increase in indicator produces only a slight decrease in the value. Part B shows that the chloride value increases progressively with increasing dilutions of the milk. The effects of dilution and the amount of indicator show that liberties cannot be taken with the details of the titration if one expects to obtain comparable results.

It is well known that this method of titrating chloride does not work

if the solution is too acid. The pH limits below which the method ceases to be reliable were determined and are shown in Part C. The acidity in one case was produced by bacterial souring, in the others by the addition of nitric acid. Acidity causes no error so long as the milk has not curdled. If the milk has curdled two procedures are open, one the neutralization of the developed acidity and the other the addition of more potassium chromate indicator. If the latter procedure is followed 0.003 or 0.006 per cent should be added to correct for the effect of increasing the amount of indicator to 3.0 or 4.0 cc. It might have been better if 3.00 cc. of indicator had been selected for the standard procedure, but in order to make all of our data strictly comparable 2.00 cc. is still used.

The use of a yellow light lowers the end point somewhat, but does not appreciably sharpen it. Adsorption indicators were much less satisfactory than potassium chromate.

#### *Morning and Evening Milking*

Samples were taken from cows which were milked three times a day; at 5 a.m., 1 p.m., and 7 p.m. Only samples from the milkings at 5 a.m. and 7 p.m. were examined. On August 5, 1933, the milk from 29 cows was titrated; in 26 pairs the morning milk was the higher by the average amount of 0.007 per cent. On August 8, 1933, the milk from 40 cows was examined; in 39 pairs the morning milk was the higher; the average difference being 0.006 per cent. The cow which was the exception gave evening milk which was 0.001 per cent higher than the morning milk. This same cow was one of the three exceptions on August 5. The longer period between milkings in the case of the morning milk may account for the higher chloride value.

Sharp and de Tomasi (30) have presented a table showing the direct titratable chloride values of morning and evening milk obtained on successive days from a number of producers. The samples obtained from each producer were quite uniform unless the chloride value was high, in which case the value fluctuated greatly. This was probably due to cows which were milked once a day, some producers milking them in the morning, others in the evening. Variations in the severity of mastitis might also have played a part.

#### *Udder Quarters and Fraction of Total Milking*

Samples from the first, middle, and last portions of milk drawn from each of the four quarters of the udders of a number of cows were titrated. Data were obtained on three groups of cows; (1) normal in mid-lactation, (2) normal at the end of lactation and (3) cows in mid-lactation having mastitis. Results typical of the three types are presented in Table 1.

It will be noted that the chloride values of the milk from normal cows in mid-lactation are relatively low, and that the differences between the first, middle and last milk and between the different quarters of the udder are approximately within the experimental error of the titration method.

The uniformity between quarters and fractions of milk drawn tends to extend to the normal milk from cows late in lactation except the chloride values are higher. Some variation in the chloride content between the different quarters would be expected in late lactation since all quarters may not dry up at the same rate. The cows in late lactation were given a physical examination by a veterinarian to determine to what extent the high chloride values might be due to mastitis. Of special interest are the left

TABLE I

*Examples of the direct titratable chloride values of the first (F), middle (M) and, last (L) portions of milk drawn from each quarter of the udders of normal, late lactation and mastitis cows*

COW NUMBER	PORTION	LEFT		RIGHT	
		Front %	Rear %	Front %	Rear %
Normal Milk					
1	F	0.099	0.101	0.099	0.101
	M	0.095	0.099	0.101	0.100
	L	0.102	0.106	0.099	0.106
2	F	0.089	0.094	0.090	0.090
	M	0.089	0.091	0.091	0.090
	L	0.091	0.092	0.093	0.090
3	F	0.099	0.098	0.099	0.101
	M	0.101	0.098	0.098	0.101
	L	0.101	0.099	0.098	0.101
4	F	0.103	0.104	0.106	0.105
	M	0.103	0.104	0.105	0.105
	L	0.103	0.105	0.105	0.104
Late Lactation Milk					
5	F	0.126	0.128	0.128	0.125
	M	0.123	0.129	0.127	0.125
	L	0.125	0.126	0.128	0.127
6	F	0.172	0.139	0.162	0.174
	M	0.128	0.133	0.134	0.159
	L	0.130	0.131	0.138	0.160
7	F	0.143	0.212S	0.131	0.138
	M	0.148	0.148	0.135	0.137
	L	0.153	0.153	0.150	0.141
8	F	0.223	0.237D	0.212	0.153D
	M	0.194	0.166	0.222	0.166
	L	0.211	0.177	0.230	0.178
9	F	0.186S	0.165S	0.174S	0.194S
	M	0.157	0.157	0.143	0.148
	L	0.139	0.134	0.144	0.148
10	F	0.192	0.187	0.217	0.185
	M	0.192	0.187	0.200	0.187
	L	0.199	0.189		0.188

TABLE I—(Continued)

COW NUMBER	PORTION	LEFT		RIGHT	
		Front %	Rear %	Front %	Rear %
Mastitis Milk					
11	F	0.166	0.183	0.235	0.181
	M	0.129	0.166	0.177	0.141
	L	0.142	0.197	0.257	0.161
12	F	0.136	0.182	0.139	0.114
	M	0.115	0.141	0.132	0.115
	L	0.126	0.153	0.142	0.125
13	F	0.275	0.256	0.187	0.295
	M	0.121	0.118	0.116	0.120
	L	0.132	0.119	0.119	0.122
14a	F	0.143	0.126	0.101	0.100
	M	0.105	0.104	0.098	0.098
	L	0.126	0.122	0.100	0.100
14b	F	0.170	0.127	0.098	0.101
	M	0.104	0.102	0.095	0.095
	L	0.115	0.112	0.094	0.096
14c	F	0.132	0.110	0.107	0.109
	M	0.107	0.108	0.100	0.102
	L	0.130	0.121	0.101	0.103

S = slight mastitis. D = distinct mastitis.

front and right front quarters of cow number 8. The chloride was higher in these two quarters than in the other two, yet these quarters did not show evidence of mastitis, while the other two showed distinct evidence of it. The chloride content, even though high, was fairly uniform in the three parts of the milk drawn from these two non-mastitis quarters.

The cows in the mastitis group were in the middle of lactation. The milk from cow 14 was titrated on three successive days, as indicated by a, b, and c. Right front and rear quarters were free from mastitis, the other two showed evidence of it. The chloride values in the two mastitis quarters fluctuated considerably from day to day and in the different portions of the milk.

Ordinarily the first and last milk drawn from quarters infected with mastitis is high in chloride and pH, and low in titratable acidity, while the middle milk is often normal in chloride, pH and titratable acidity. A few exceptions were found when production was very low and when the milk was stringy. This lack of uniformity usually permits one to distinguish cows giving milk high in chloride due to mastitis from those giving high chloride due to late lactation. The fraction of the milk drawn is important in testing for mastitis. In several of the mastitis group of cows, if the first milk drawn were tested, very severe mastitis would be indicated, while if the middle milk alone were examined the report might be that the chloride, pH, and titratable acidity tests for mastitis were negative. The first streams should be selected in testing for mastitis.

*Stage of Lactation*

During the years 1932 and 1933 the milk from a number of healthy cows was titrated monthly for chloride. In the group were included 13 Jerseys, 8 Guernseys, 15 Holsteins, 4 Brown Swiss, 5 Ayrshire and 4 Short-horns. In all 326 samples were examined. The cows were examined regularly by veterinarians and all samples from cows showing active mastitis were eliminated. Furthermore if the chloride values began to increase unexpectedly, and later active mastitis was recognized, the data from such cows were excluded. This observation in regard to the detection of mastitis agrees with that of Rosell (27) who found that the early stages can be detected best by chemical and less readily by bacteriological means. In Figure 2 the titratable chloride values are plotted against the fraction of the total lactation period.

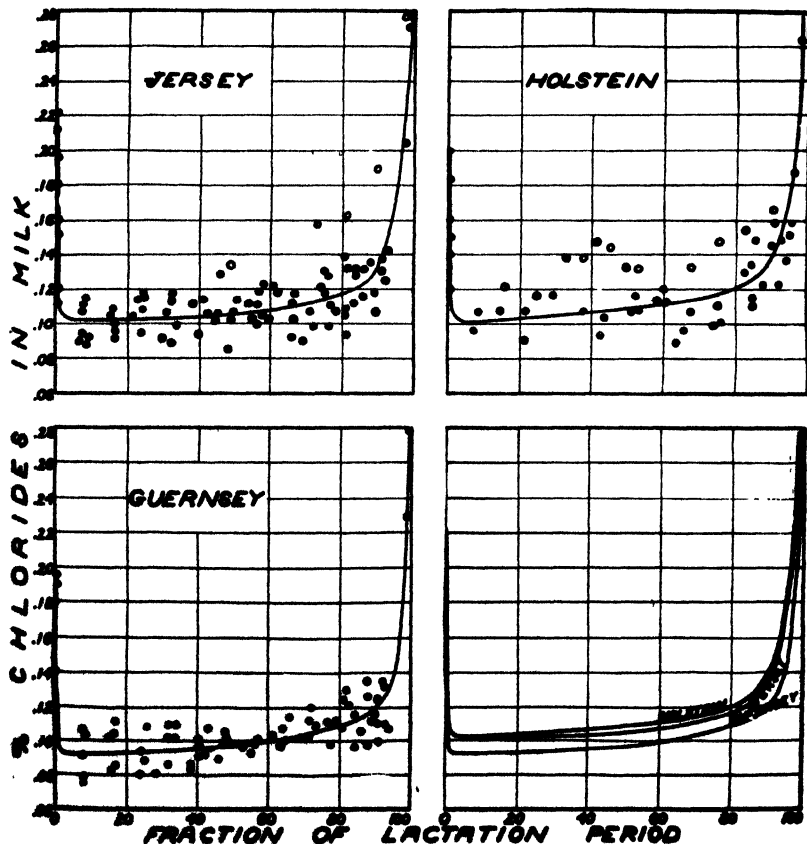


FIG. 2. Fraction of the lactation period and the direct titratable chloride value of the milk of different breeds.

Colostrum milk gave quite high chloride values, but the value fell rapidly so that at the end of one to two weeks a minimum was reached. The chloride increased very slowly during the next 60 per cent of the lactation period and from there on more rapidly until a very abrupt rise occurred after 90 per cent of the period of lactation had been completed. The data indicated by circles were obtained from one or two cows. These cows were repeatedly pronounced free from mastitis. They were outstandingly hard milkers.

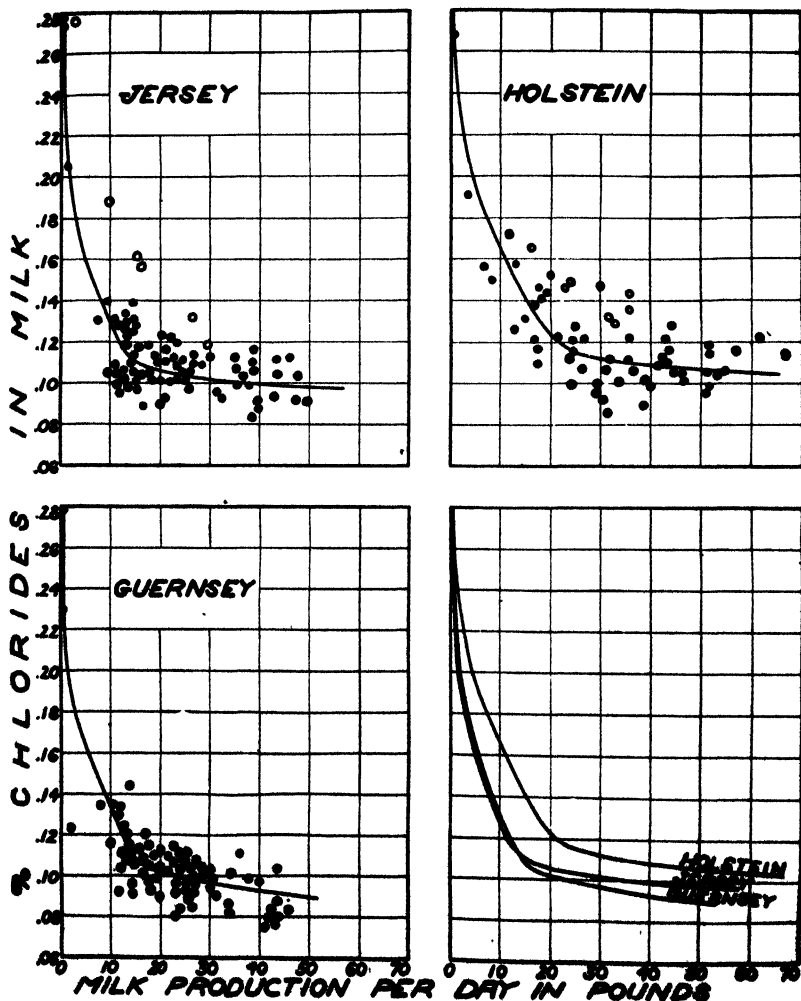


Fig. 3. Daily production of milk and the direct titratable chloride value of the milk of different breeds.

### *Production*

In Figure 3, the direct chloride titration values are plotted against the average daily production. The results show a striking relation between the two factors. The chloride values begin to rise rapidly after the production falls below about 15 pounds for the Jerseys and Guernseys, and below about 25 pounds for the Holsteins. This relationship is of service as indicating whether high chloride values are due to late lactation or to a diseased condition in the udder. The importance of the relation to production was shown by a Holstein cow which still gave about 25 pounds of milk after being milked continuously for about five years. The chloride value was 0.13 per cent.

### *Breed Difference*

The data indicate quite clearly that Holstein milk tends to be higher in chloride than does Guernsey or Jersey milk. This is probably a true breed difference and is not due to a systematic error, since the known errors point in the reverse direction. The interfering proteins are higher in the Guernsey and Jersey milk. The cows had all been scored for severity of mastitis according to a system devised by Udall and Johnson (35) on a basis of 1 to 4, the score 1 indicating little or no evidence of mastitis and 4 indicating the active condition. According to this classification the average scores were as follows: Guernsey 1.96, Jersey 1.93 and Holstein 1.73. Sharp (29) reported a correlation of  $-0.603 \pm 0.013$  between titratable acidity and titratable chloride content of over 1000 samples of market milk. This correlation is in line with the breed difference, but was undoubtedly influenced by the presence of milk from cows late in lactation and having mastitis.

### *Effect of Gestation*

The effect of gestation on the chloride value is shown in Figure 4. The chloride is plotted against the months of gestation in Part D, and the expected increase with gestation is shown. The same data are plotted against months of lactation and give a curve which is more irregular because of the variation in the lactation periods. The curves in Part B indicate that although the chloride content increases as the lactation period advances, the progress of gestation tends to negate, to some extent, the increase in titratable chloride. This effect is also indicated in Parts A and C. At the same level of production the chlorides are lower the more advanced the period of gestation. This result suggests the possibility that the chloride content of the blood of the cow might decrease as the gestation advances. Such a decrease in chlorides in the blood would be in line with the increase in blood volume as found by Miller (14). Other explanations such as a change in the secreting mechanism in the udder are possible.

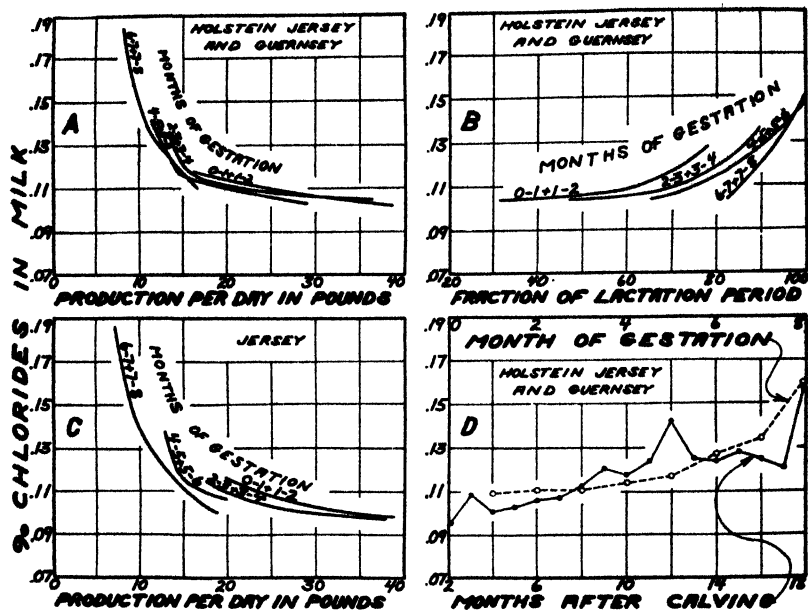


Fig. 4. Period of gestation and the direct titratable chloride value of the milk.

#### SUMMARY AND CONCLUSIONS

The following statements refer to the chloride value of milk as determined by the direct titration of the milk with silver nitrate, using potassium chromate as an indicator.

1. The direct titratable chloride value of milk was found to increase with the dilution of the milk and with decreased amounts of indicator. As the acidity of the milk increased, no influence upon the end point was found until after the casein had coagulated.
2. Differences between normal morning and evening milk were slight.
3. The fraction of the total milking of the quarter of the udder and the different quarters themselves had little effect on the chloride values of the normal milk from healthy cows.
4. The first and last milk drawn from quarters infected with mastitis was high in chloride and pH and low in titratable acidity, the middle portion often approached or fell within the range of normal mastitis-free milk. Wide variations were found between the different quarters in the case of cows infected with mastitis.
5. The chloride in milk from healthy cows fell very rapidly during the first few days of lactation, reached a minimum, increased slightly during the first 60 per cent of the lactation period, and then increased more rapidly, particularly during the last 10 per cent of the period.

6. A definite relation was found between the chloride value and the daily production of milk from healthy cows. A marked increase in chloride began when the production of Jersey and Guernsey cows fell below 15 pounds, and when the production of Holstein cows fell below 25 pounds of milk a day.

7. The milk from healthy Holstein cows tends to be higher in chloride than does the milk from healthy Jersey and Guernsey cows.

8. Gestation exerts a negating tendency on the increase in chlorides due to progressive lactation.

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# THE ADVANTAGES OF SKIM-MILK AGAR FOR THE DETERMINATION OF THE SANITARY QUALITY OF MARKET MILK

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Attention was called by Sherman (1) to the advantages in using an agar containing a fermentable carbohydrate in the bacteriological control of market milk. He presented data which showed that the colonies of bacteria normally present in milk were two to ten times larger on lactose agar than on plain agar. He made comparative bacterial counts on plain and lactose agar which showed an average increase, in favor of lactose agar, of 43 per cent on raw milk and 378 per cent on pasteurized milk. Sherman mentioned that "glucose is apparently just as efficient as is lactose, both as to number and size of colonies," and that "since glucose is cheaper it should perhaps be recommended as the standard for routine work." He also suggested that the amount of sugar could be reduced to 0.1 per cent "without impairing the value of the medium." Simmons (2), and others, have confirmed Sherman's findings.

Ayers and Mudge (3) suggested a milk powder agar for use in determining the numbers and kinds of bacteria in market milk and other dairy products. They found that milk powder agar gave from 3 to 75,000 per cent higher counts than those obtained on plain agar. The colonies were larger and consequently could be counted with greater ease and accuracy. The casein agar reported by Ayres (4) was found to be unsatisfactory unless incubation periods longer than two days were employed.

The modification of milk powder agar suggested by Zoller (5) was found by Norton and Seymour (6) to be a definite improvement. This modified milk powder agar gave higher counts than any other medium tested by these workers, but it was criticized because of the complicated procedure involved in its preparation.

## EXPERIMENTAL

The results here reported include data obtained on approximately 760 samples of milk taken from the milk supply of 250 dealers located in various parts of the State of New York. These milk samples were taken three times during the year at periods representing winter, spring, and summer conditions. The samples of milk were plated in duplicate, in dilutions of

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1-100 and 1-1,000, on standard nutrient agar and on a milk agar having the following composition:

Fresh raw skim milk	2.0 per cent
Bacto peptone	0.5 per cent
Bacto beef extract	0.1 per cent
Glucose	0.1 per cent
Agar	1.5 per cent

This medium is a modification of one employed with good results by J. M. Sherman (unpublished data) almost twenty years ago, in which he used 0.2 per cent skim milk powder, 1 per cent peptone, and 1.5 per cent agar. In the medium here reported, skim milk was used instead of milk powder with the belief that it would be more uniform in consistency, freer from sediment, and would give uniformly high bacterial counts. Small amounts of beef extract and glucose were added on the assumption that they might improve the nutritive value of the medium, and support the luxuriant growth of certain types of bacteria which, in the absence of these constituents, might grow very poorly or not at all. A medium of the above composition is simple, easily made, and should cost as little or probably less than the present plain agar suggested by Standard Methods.

Ayers and Mudge (3) say "attempts to make a medium by using skimmed milk, with its casein dissolved, and adding peptone and beef extract were not successful." If much larger amounts of skim milk, or phosphate buffers, are added to the medium, precipitation will occur. The required amounts of glucose and skim milk may be added to the adjusted, autoclaved, and filtered nutrient medium just previous to its final sterilization. If the medium previous to the addition of milk is adjusted to pH 7.0, the reaction of the completed medium will be approximately pH 6.5. If thought advisable, the pH could be raised by the addition of sodium hydroxide.

Table 1 shows the general trend of the results obtained from the comparative counts made on the three sets of samples. These data are reported

TABLE 1  
*Average percentage increase in favor of skim-milk agar between counts made on standard agar and on skim-milk agar*

KIND OF MILK	WINTER SAMPLES FEB 6-7		SPRING SAMPLES MARCH 28-29		SUMMER SAMPLES JUNE 6-7	
	No. tested	Per cent increase	No. tested	Per cent increase	No. tested	Per cent increase
Pasteurized ..	221	127	199	351	198	196
Raw ....	25	5	56	16	56	18

separately because of the extreme differences in temperature when the tests were made. Many extremely high counts were observed during the warmer

weather. Frequently neither set of plates could be accurately counted on the highest dilution used, which probably tended to obscure many of the existing differences in counts. On the average, the skim-milk agar count on pasteurized milk was two to four times as large as the corresponding count on standard agar. A slight increase in favor of skim-milk agar was also observed on the smaller number of samples of raw milk tested.

The presence in pasteurized milk of certain bacteria which are unable to grow on standard agar is the obvious reason for higher counts on skim-milk agar. That varying numbers of such bacteria are often found in pasteurized milk is well known. Probably the presence of these types of bacteria explains the results reported in Table 2.

TABLE 2

*Some striking differences in counts from pasteurized milk on standard agar and on skim-milk agar*

NUTRIENT AGAR COUNT	SKIM-MILK AGAR COUNT	DIFFERENCE IN FAVOR OF SKIM-MILK AGAR
		<i>Per cent</i>
7,900	1,230,000	15,470
2,400	73,500	2,962
29,900	815,000	2,626
2,100	57,300	2,152
5,600	46,500	730
14,900	117,000	685
19,500	119,000	510
28,500	123,000	332
2,400	9,300	287
3,500	10,300	190

It will be observed from the distribution of percentage differences reported in Table 3 that 60 per cent of the 608 pasteurized milk samples showed a definitely higher count on the skim-milk agar; about 35 per cent

TABLE 3

*Relative distribution of percentage differences in counts from pasteurized milk on plain agar and skim-milk agar, using plain agar as standard*

RANGE IN PERCENTAGE	NUMBER OF SAMPLES
- 26 per cent to - 60 per cent	28
- 25 per cent to - 1 per cent	70
0 per cent to 25 per cent	145
26 per cent to 200 per cent	274
201 per cent to 500 per cent	50
501 per cent to 2000 per cent	33
2001 per cent to 5000 per cent	5
Over 5000 per cent	3
Total ...	608

gave approximately the same count ( $-25$  per cent to  $+25$  per cent); and less than 5 per cent of the samples tested gave a lower count on the skim-milk agar. It is obvious that the difference between the counts on different samples is far from consistent. This very inconsistency brings out most clearly the advantages of the skim-milk medium.

Since logarithms of bacterial counts tend to minimize small differences which might be considered significant and also offer an opportunity to present large groups of data in a small space, the logarithms of the bacterial counts for each period have been plotted in Figures I, II, III, and IV.

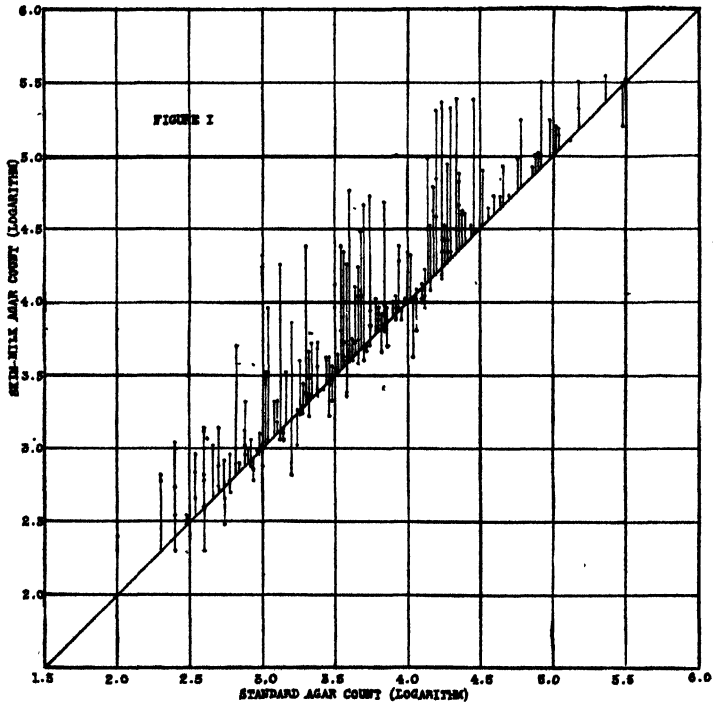


FIG. I. The logarithm of the number of bacteria in *pasteurized milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken February 6 and 7.

If the skim-milk agar count exceeds the plain agar count, its logarithm appears above the diagonal line; if it is less, it appears below this line. The distance vertically from each dot to the diagonal line represents a single logarithmic difference. In certain cases, several of these differences are plotted on the same vertical line. This means that the standard agar counts

on several samples were the same, whereas the skim-milk agar counts varied. Each dot represents one skim-milk agar count. The logarithms of these comparative counts on pasteurized milk appear in Figures I, II, and III. A glance at these graphs shows that the bulk of the skim-milk agar counts are higher than the standard agar counts. The logarithmic differences on certain samples are conspicuous. The general trend of the three charts is similar, although the counts on both media tend to be higher as warmer weather approaches.

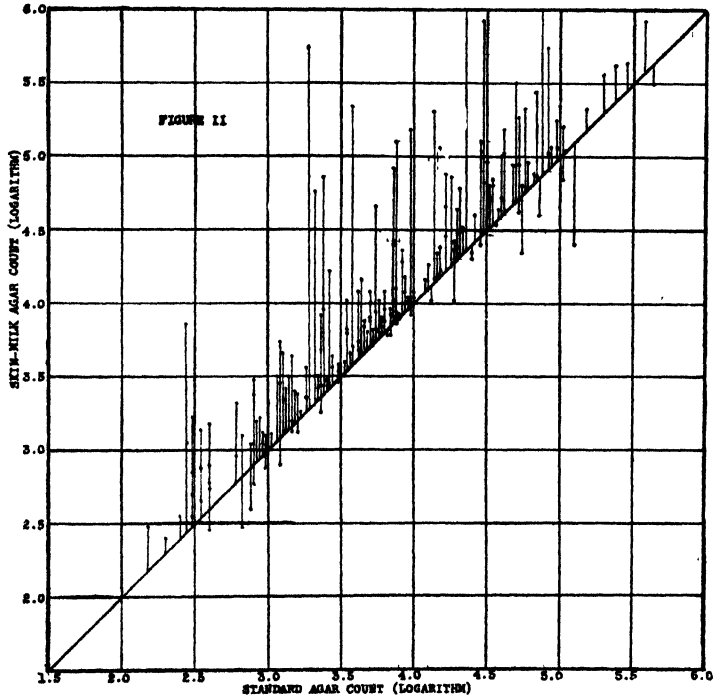


FIG. II. The logarithm of the number of bacteria in *pasteurized milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken March 28 and 29.

Because of the small numbers of raw milk samples tested, the results for the three periods are presented together in Figure IV. The logarithmic differences in these counts on raw milk are believed to be of but little significance.

These data have been analyzed according to the percentage difference in counts, based on logarithmic averages as employed by Robertson and Frayer (7). The logarithmic averages of the standard and skim-milk agar

counts on pasteurized milk and the percentage differences calculated from these averages are presented in Table 4. Similar calculations were made

TABLE 4  
*Theoretical percentage differences in counts from pasteurized milk based on logarithmic averages*

	LOGARITHMIC AVERAGES		DIFFERENCE IN COUNTS : PERCENTAGE
	SKIM-MILK AGAR COUNTS	STANDARD AGAR COUNTS	
Winter samples Feb. 6-7 . . .	3.787	3.585	59
Spring samples Mar. 28-29 . .	4.024	3.804	66
Summer samples June 6-7 . . .	4.616	4.366	78

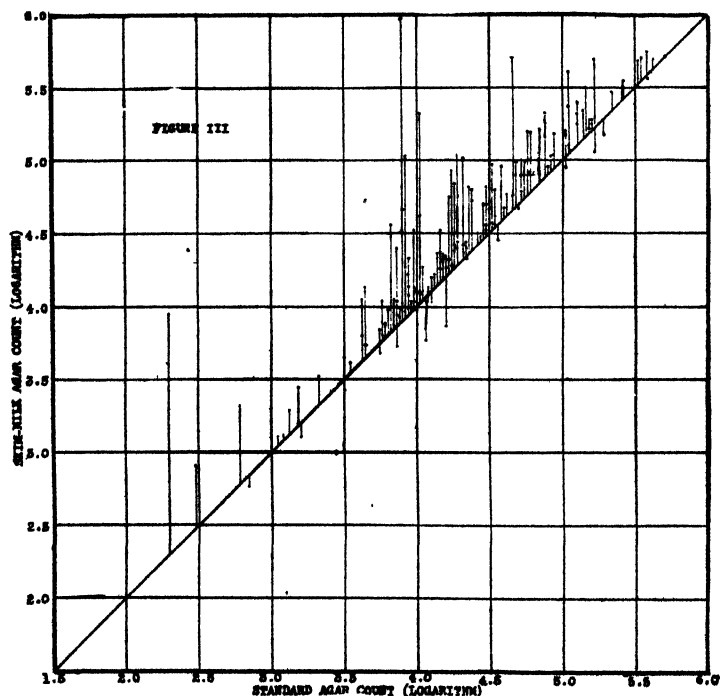


FIG. III. The logarithm of the number of bacteria in *pasteurized milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken June 6 and 7.

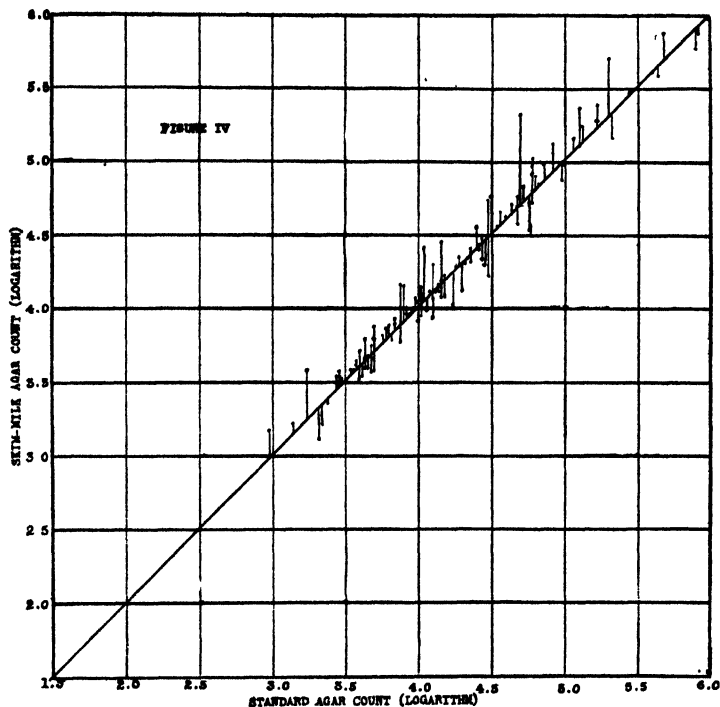


FIG. IV. The logarithm of the number of bacteria in raw milk which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken February 6 and 7; March 28 and 29; and June 6 and 7.

on the raw milk data, but were found to have but little significance. For each set of logarithmic averages the percentage difference in count was calculated from the theoretical plate counts corresponding to these averages. This is illustrated for the spring samples of pasteurized milk:

Logarithmic average of skim-milk agar count is 4.024,  
which is equivalent to 10,570 bacteria per cc.

Logarithmic average of standard agar count is 3.804,  
which is equivalent to 6,370 bacteria per cc.

$$\frac{10,570 - 6,370}{6,370} \times 100 = 66 \text{ per cent theoretical percentage difference in counts.}$$

So great a percentage difference in counts, when based on logarithmic averages, is believed to be truly significant.

## SUMMARY

The fermentable carbohydrates and other milk constituents in skim-milk agar make it a desirable medium to use in the routine control of market milk and other dairy products.

The skim-milk agar counts on 618 samples of pasteurized milk were, on the average, two to four times as large as the corresponding counts on standard agar. The counts on 137 samples of raw milk were only slightly higher.

The colonies were much larger and consequently could be counted with greater ease and rapidity.

The slight opacity of the medium prevents the glare often experienced when artificial lighting devices are used. Acid-producing and protein-digesting types of bacteria can be differentiated on this medium.

It supports the growth of bacteria responsible for mastitis in cows.

It is simple and easy to make and no more expensive than the present standard agar.

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## VITAMIN A CONTENT OF PASTURE PLANTS

### 11. TIMOTHY (*PHLEUM PRATENSE* L.) AND RED TOP (*AGROSTIS ALBA* L.) UNDER PASTURE CON- DITIONS AND FED GREEN\*

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The public constantly demands higher quality in dairy products, and progressive dairymen attempt to meet such demand. In recent years, because of the consumer's interest in the vitamin content of milk, investigators, and even some commercial milk producers, have tried by various methods to fortify the vitamin content of milk. After studying the problem, Krauss (12) summarized the situation as follows: "Vitaminization and mineralization of foods probably can not be justified except where natural foods fail to furnish these vital factors. This is especially true of milk."

Although there is considerable sentiment against making milk a "drug store product" by adding material to it, nevertheless, public demand will insure continued interest in milk of high vitamin content whether produced from natural feeds or treated in some manner.

Milk, butter, and cheese are important sources of vitamin A in the human diet. Many investigators have suggested that the vitamin A content of milk might be greatly influenced by the vitamin A content of the feeds consumed by the cow. In a previous paper from this station (23) some of the earlier results published on this subject were reviewed. Later investigations (1, 3, 4, 6, 13, 14) have confirmed this conclusion. It has been shown by Moore (19) that the cow is capable of converting the carotene in her ration into vitamin A, but the daily output of carotene and vitamin A in the milk fat is very small compared with the carotene intake, never exceeding that of normal summer butter regardless of the amount in the feeds consumed. Baumann, Steenbock, Beeson, and Rupel (2) report: "When the carotene intake of the cow was increased, there was an increase in both the carotene and vitamin A content of the butterfat. Calculations indicated that 3.3 per cent of the vitamin A ingested on a low carotene ration was secreted into the milk; on a high carotene ration only 1.3 per cent was secreted."

In the past, vitamin A has not been considered a limiting factor in the well-being of cattle, but more recent results indicate that under some conditions it may be. Hart and Guilbert (9) report vitamin A deficiency in range cattle under natural conditions. Deficiency developed when the dry

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feed season was unusually prolonged. Manifestation was more severe when the diet was otherwise complete and supplied in amounts above maintenance. Reproductive failure and dead, weak, or sick calves were the most common results.

Meigs and Converse (15) conclude from their experiments that "In the case of liberally milking cows fed on grain and hay or on grain, hay, and silage, without pasture, the ration is likely to be deficient in vitamin A unless it contains a large proportion of legume hay of good quality. Young calves from birth to the age of six months are highly susceptible to A deficiencies." The same authors (14) report that when timothy hay of mediocre or low quality was fed to dairy cows reproduction was reduced, general health of the cows lowered, milk production decreased, and pregnancy was produced with more difficulty.

Woodward and Nystrom (24) in their bulletin on feeding dairy cows, point out that vitamin A is the vitamin most likely to be deficient in the ration of the dairy cow, and that as a result of this deficiency cows may give birth to weak, dead, or premature calves. They believe the quantity of milk will not be reduced so much as the vitamin A value. Because of the reserve supply in the liver, the cows themselves may go for months without showing the bad effects of the deficient ration, but calves fed the milk from such cows will cease to grow and soon will die if they are not given supplementary feeds rich in vitamin A or carotene.

Storage of vitamin A in the body of the cow may be sufficient to take care of other body functions for several months, but Hilton, Hauge, and Wilbur (11) found that as far as milk production is concerned the vitamin A value of butter responds very rapidly to changes in the vitamin A value of the rations fed to the cows and that it is possible to maintain a high vitamin A value in butter by practical feeding methods. Fraps, Copeland, and Treichler (8), however, from their study of the vitamin A requirements of dairy cows conclude that : "silage and ordinary hays and fodders apparently will not supply enough vitamin A potency to maintain a high content of the butterfat. Green growing pasture grasses appear to be needed to maintain the production of butterfat high in vitamin A."

Dutcher (5) calls attention to the fact that the vitamin content of leafy plants and vegetables may be correlated with greenness, metabolic activity, and maturity. In discussing the growth response from vitamin A and its precursor, carotene, he sums up the problem as far as animal feeding is concerned as follows: "It is probable that we shall find that some of our so-called 'vitamin A-rich' foods are really quite deficient in this vitamin, *per se*, but that these foods are really rich in the parent substance, carotene. From the nutritional and clinical standpoints this fact need not cause concern so long as the fat-soluble factor is rendered available for the animal organism."

Investigation of the vitamin A activity of the most common pasture plants seems justified because, first, the vitamin A activity of the feed consumed by the cow affects the vitamin A activity of the milk and butter, which is important in human nutrition; second, pasture represents the major portion of the cow's feed during nearly half the year; and third, significant differences in the vitamin A activity of different plants may indicate consideration of this factor along with others in formulating pasture mixtures for dairy cows.

Very little has been reported on the vitamin A activity of forages. In a previous paper from this Station (23), the vitamin A activity of white clover (200 rat units per gram) was reported to be about twice that of Kentucky bluegrass.

#### EXPERIMENTAL PROCEDURE

The method used for determining the vitamin A content of these plants was based upon the technique of Sherman and Munsell (20). Young albino rats, 21 to 28 days old and weighing from 32 to 51 grams, were taken from mothers who had received a ration consisting of 62 per cent ground whole wheat, 30 per cent whole milk powder, 4 per cent wheat germ, 3 per cent powdered egg yolk, and 1 per cent iodized salt. These rats were placed on a basal vitamin A-free diet which consisted of 67 per cent cornstarch, 18 per cent air-heated casein, 10 per cent dried yeast powder, 1 per cent sodium chloride, and 4 per cent salt mixture (Osborne and Mendel (17)).

To free the ground commercial casein of vitamin A, it was treated by the heat method suggested by Potter (18). Three hundred gram quantities were spread on shallow trays to a depth of  $\frac{3}{4}$  inch and heated in a Freas oven at 110° C. for seven days. The casein was stirred twice daily to secure better exposure. After heating it was washed twice with distilled water and allowed to dry. Vitamin D was furnished by feeding one drop of Mead's Viosterol three times a week.

At the end of three weeks of the depletion period the rats were put in individual cages made of  $\frac{3}{4}$  inch wire screen. From the time the rats were put in individual cages to the end of the depletion period they were weighed every other day.

As soon as there was a cessation of growth or the first sign of ophthalmia (slight swelling of the eyelids and an accumulation of exudate on the cornea), usually accompanied by a barely perceptible tendency to flabby musculature and slightly unkempt fur, the depletion period was ended and the test period begun.

Fresh green samples of timothy and red top under pasture conditions were received twice a week from the Caldwell Substation. To prevent moisture losses in shipping, the grass was wrapped in cellophane or paper sacks and placed in paper cartons. Upon arrival, each sample was placed

in a half-pint Economy fruit jar and kept in the refrigerator. Practically all the samples consisted of basal foliage and contained very few stems. Timothy had an average moisture content of 68 per cent for all feedings, while red top contained 61 per cent moisture.

The grasses were fed three times a week as supplements to the vitamin A-free diet. Timothy was fed at the rate of 10 and 12½ milligrams per day, while red top was fed at the rate of 10 and 7½ milligrams. Litter mates were used for comparisons between grasses, and a negative control from each litter was continued on the basal diet until death.

Weekly records of weights and food consumption, and careful notes on the condition of the animals were kept for eight weeks. The method of eye scoring was that used by Steenbock and Wirick (22). All animals were autopsied at death or at the end of the experimental period.

### RESULTS

At the beginning of the depletion period, the average weight of the rats in all groups was 42 grams, and at the end of the depletion period 98 grams. The average length of the depletion period was 26 days.

Average growth response of the rats being fed each of the grasses is shown in Table 1 and Figure 1. The experiments here reported were planned and carried out on the basis of the eight-week test period used by Sherman and Munsell (20), but results are presented for periods of four weeks (28 days). A 1934 revision of the "Text and Assays for Cod Liver Oil" of the Pharmacopoeia of the United States prescribes an official method of assay for vitamin A (16). Specifications for the depletion period, assembling of rats in groups, and recording of data are given. Concerning the validity of data the method reads as follows: . . . "The data from an assay group shall be considered valid for establishing that an assay oil conforms with the U. S. Pharmacopoeia standard for Vitamin A in cod liver oil only when two-thirds or more but not less than six rats shall have made individually between the beginning day of the assay period and the twenty-eighth day thereafter an increase in body weight which shall equal or exceed 12 grams." This rule has been used to establish the validity of data from an assay group for vitamin A potency of pasture plants. No rat which has not made a gain of 12 grams or more in four weeks has been used to establish average performance of an assay group. The number of rats in each group and the number used in the average are indicated in the table. The results can not be expressed in International Units of Vitamin A; however, because there are no data from a group fed a "Reference Cod Liver Oil." The value is calculated, therefore, in rat units per gram from the daily dose in milligrams which would give an average gain of 12 grams in 28 days.

Animals (10 rats) receiving 60 milligrams of timothy per week made

TABLE 2  
Average growth response of rats when fed fresh timothy and red top as vitamin A supplements

PASTURE PLANT	SOURCE	DATE OF TEST PERIOD	AMT. OF PLANT FED WEEKLY	RATS FED	RATS USED	Ave. wt. at beginning of test period	Ave. wt. at ending of 4 weeks test period	Ave. gain in wt. at 4 weeks	Coefficient of variation	Rat units of vitamin A
Timothy	Caldwell	5-16 to 7-11	<i>gms.</i> 0.060	12	10	<i>gms.</i> 43	<i>gms.</i> 126	<i>gms.</i> 26 ± 1.5	26	220 ± 13
"	"	"	0.075	4	4	39	124	23	*	*
Red Top	"	"	0.060	12	10	42	135	37 ± 1.1	15	308 ± 10
" "	"	"	0.045	4	4	39	124	25	*	*
Negative Controls	"	"	0.000	8		43			average survival—15 days average loss in weight—15 grams	

\* Insufficient data.

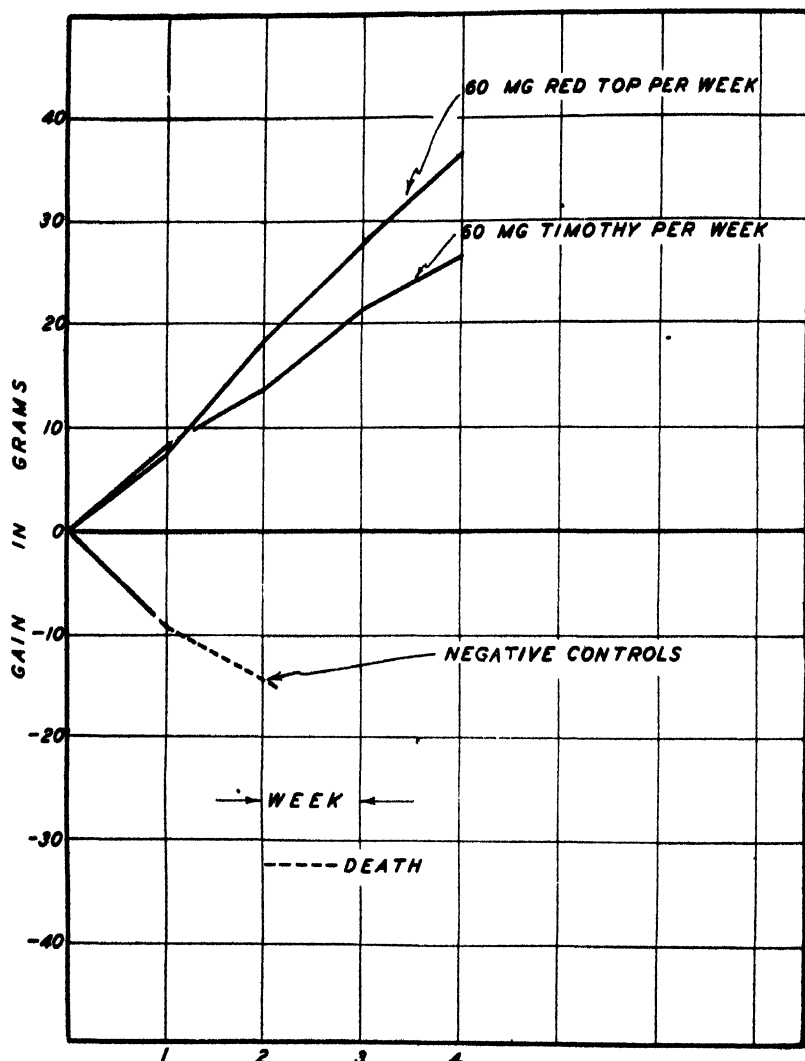


FIG. 1. Average weekly weight increases of rats, previously depleted of vitamin A, when fed either timothy or red top as a supplement to a vitamin A free diet.

Average weekly losses in weight of negative control rats were computed each week by averaging the weight of all living rats as long as 50 per cent of the original group survived. The line was terminated by extending it directly from the last week showing 50 per cent survival to a point indicating the average survival days and average weight at death for the entire group. The broken line indicates that one or more deaths occurred during the week.

an average total gain in body weight of  $26 \pm 2$  grams in four weeks. This would indicate that this grass contained  $220 \pm 13$  rat units of vitamin A activity. The average total gain of the 10 rats fed 60 milligrams of red top per week was  $37 \pm 1$  gram. This would indicate  $308 \pm 10$  rat units of vitamin A activity per gram in red top.

The coefficient of variation in the group fed timothy, 60 milligrams per week, was 25 per cent, while in the group fed red top, at the same level, was 16. The variability of these results compare favorably with the findings of Sherman and Burtis (21), who report that when the animals were classified by sexes the coefficient of variation was 32 per cent at the end of the fifth week at a vitamin intake level twice that needed for a growth response of 3 grams per week and 45 per cent at a level inducing 3 grams gain per week.

At the other level on each grass, the great variation found within the group, together with the limited number of rats (less than six), would preclude conclusions from these groups other than to substantiate the dependability of the levels used in interpreting results.

The difference between the rat units of vitamin A activity in red top, 308 units, and timothy, 220, was 88 units. The probable error of the difference was 11 units, or the reliability of the difference may be expressed as  $88 \pm 11$ . Since the difference between the rat units in the two pasture plants (88) was eight times the probable error of the difference, the difference is a true one.

In order that the results obtained may be compared with data reported on the basis of 24 grams gain in eight weeks, the average gain made by each group in eight weeks is given. When the average gain for the longer period is the basis used for calculating the rat units per gram, all animals in a group are usually included whether or not they are gaining at the end of the test. All animals, therefore, are used in summarizing the eight-week averages. On the 60-milligram per week level the 12 animals fed timothy gained  $39 \pm 1.2$  grams in eight weeks, indicating a value of  $163 \pm 5$  rat units per gram; while the 12 rats on red top gained  $48 \pm 3.4$  grams, or  $200 \pm 15$  rat units per gram. All the animals receiving 60 milligrams of red top per week were free of xerophthalmia at the end of four weeks and also at end of eight weeks. Only one of the animals of the group used with the timothy showed eye infection at four weeks, and this condition failed to clear by the end of eight weeks. One of the other rats included in the longer test group on timothy also failed to develop normal eyes. Post-mortem examinations of the animals at the end of eight weeks revealed some signs of infection, especially in the ear and nasal sinuses. The eight animals used as negative controls survived an average of 15 days beyond the end of the depletion period and lost an average of 15 grams in body weight. Post-mortem examination of these animals showed that 3 had pus in the

nasal sinuses, all had pus in the inner ear, 2 had pus at the base of the tongue, 2 had congestion in the intestinal tract, and 1 had pus in the lymph glands.

#### DISCUSSION

White clover and Kentucky bluegrass previously have been reported to contain 200 and 100 rat units, respectively, of vitamin A activity when computed from data covering a feeding period of eight weeks (23). When computed from gains made in the first four weeks, as used in this paper, the white clover contained  $242 \pm 19$  rat units and the Kentucky bluegrass  $175 \pm 11$ . In comparable units, timothy with  $220 \pm 13$  would rank between bluegrass and white clover. Red top with  $308 \pm 10$  units contained about one-third more than even white clover. These results indicate that different pasture plants may vary considerably in their content of vitamin A activity. Although the 4 weeks test period seems to have some advantages over the 8 weeks period, it appears that higher vitamin A rating may be expected from the same feed level.

The fact that red top contained about 50 per cent more rat units of vitamin A activity than timothy may be attributed to the possibility of a higher carotene content. Although no tests for carotene were conducted, and each grass was green when fed, some justification for this assumption is indicated by the fact that red top takes on a reddish cast when mature while timothy does not.

Although many writers have suggested that fresh green grass is probably a potent source of vitamin A no other bio-assays have come to the notice of the writers. Fraps and Treichler (7) reported that samples of bur clover and sudan grass dried in vacuum contained 200 and 150 units per gram, respectively, but no data were presented on the fresh green grasses as the cows consume them.

Any loss of moisture would tend to concentrate other constituents in the grass samples and would probably indicate a higher vitamin A content than the original grass contained. Although some moisture was lost from the samples studied, the grass was still fresh and green when fed. The average moisture content of the timothy samples was 67.9 per cent and of the red top 60.5. These averages compare well with the average of 60.7 per cent for 16 analyses of red top and 62.5 per cent for 88 samples of timothy, all analyses, and 75.8 for 5 samples, before bloom, reported by Henry and Morrison (10).

The vitamin A activity of each of these four pasture plants is much higher than has been reported for most feeds or foods except some samples of fish liver oils.

Fraps, Copeland, and Treichler (8) were unable to maintain high vitamin A activity in butter when cows were fed 116,000 rat units daily. They

suggested that fresh green grass was necessary in the diet of the cow in order to keep the vitamin content of the butter high.

A dairy cow can easily consume 100 pounds of fresh green grass daily. If the grass contained 200 rat units of vitamin A activity per gram, the total intake of vitamin A activity per day would be over nine million rat units. It is not surprising that summer butter produced by cows fed unlimited quantities of fresh grass has been found to be usually higher in vitamin A activity than winter butter.

#### CONCLUSIONS

Results of this study indicate that the vitamin A activity of the two pasture plants, timothy (*Phleum pratense* L.) and red top (*Agrostis alba* L.), was  $220 \pm 13$  and  $308 \pm 10$  rat units respectively, considering a gain of 12 grams in 4 weeks as representing one unit. Statistically, this is a significant difference. Compared with white clover ( $242 \pm 19$  units) and Kentucky bluegrass ( $175 \pm 11$  units) previously reported, timothy would rank between them while red top contained a third more units than even white clover. The content of vitamin A activity in these pasture plants is higher than has been reported for any feeds or foods.

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# STUDIES ON THE CHEMICAL COMPOSITION OF THE BLOOD OF DAIRY CATTLE

## I. THE EFFECT OF AGE AND PHOSPHORUS INTAKE ON THE CALCIUM AND INORGANIC PHOSPHORUS CONTENT OF WHOLE BLOOD OF DAIRY HEIFERS

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The calcium and inorganic phosphorus content of the blood has been studied rather extensively in recent years in the diagnosis of phosphorus deficiency and in studies of mineral metabolism and disease.

In a study of the effect of low-mineral rations on the growth and development of dairy heifers considerable data have been obtained on the effect of age and phosphorus intake on the calcium and inorganic phosphorus content of the whole blood of growing dairy heifers.

The inorganic phosphorus is one of the constituents of the blood that is commonly thought to be influenced by the amount of phosphorus in the diet. Many of the data leading to this conclusion have been secured on small laboratory animals. However, Meigs, Blatherwick, and Cary (15), Blatherwick (4), Robinson and Huffman (20), and others have obtained results on dairy animals supporting this assertion. Theiler, Green and DuToit (24) were the first to produce an experimental "aphosphorosis" by feeding growing heifers rations low in phosphorus. They reported 5.2 mgs. of inorganic phosphorus per 100 mls. of whole blood for normal animals while the animals on the low-phosphorus rations were as low as 1.3 mgs. per 100 mls. Palmer and Eckles (18) reported low values for inorganic phosphorus in the blood of animals raised in a phosphorus deficient region. Malan, Green and DuToit (14) have shown that the blood of heifers grown on phosphorus deficient pastures is likely to be low in inorganic phosphorus. Results obtained by Henderson and Weakley (8) and Eckles and associates (5) show that when the phosphorus in the feed is lowered the inorganic phosphorus in the blood is decreased in a relatively short time. Meigs, Blatherwick, and Cary (15), Eckles and associates (5), and others have shown that the inorganic phosphorus of the blood of animals on low-phosphorus rations may be raised to normal by supplementing the ration with sodium phosphate or by feeding normal feeds high in phosphorus.

Meigs and associates (15), Palmer and Eckles (17), and Henderson and Weakley (8) have reported age as a factor affecting the inorganic phosphorus

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of the blood. Inorganic phosphorus was found to be fairly high in new-born calves and tended to increase, reaching a maximum at about six months of age. This maximum was followed by a gradual decline as the animals grew older.

Meigs and coworkers (15) reported the concentration of calcium in the plasma of cows' blood to be fairly constant. Palmer and Eckles (19) however found it subject to significant fluctuations of an undertermined cause in spite of a high coefficient of correlation on successive days.

Palmer and Eckles (18) obtained normal values for calcium when the inorganic phosphorus was much below normal. Supplementing the low phosphorus ration with calcium carbonate or sodium phosphate was without effect on the calcium content of blood plasma.

Results obtained by Anderson, Gayley, and Pratt (1) indicate no noticeable difference in the calcium of the blood of young and mature animals. However, Henderson and Weakley (8) and Huffman and associates (12) have obtained some evidence that there is a slight diminution in the calcium concentration of heifers' blood with an increase in age.

With these points in mind it was thought desirable to make a further study of the effect of age and level of phosphorus intake on the calcium and inorganic phosphorus content of the blood of dairy heifers during the period of growth and to continue this study through the periods of gestation and first lactation. It seemed probable that much information might be gained as to the minimum requirement of phosphorus for such animals. This report covers the period of growth from birth to about 25 months of age.

#### EXPERIMENTAL

For this study three groups of grade Holstein heifer calves were used. One group was fed a normal ration while the others were fed rations low in phosphorus.

The normal group consisted of seven calves and was fed a ration composed of timothy hay, corn meal, ground oats, wheat bran, and corn-gluten meal. The calcium and phosphorus were assumed to be present in sufficient amounts, as the ration is representative of those fed under practical farm conditions without any apparent harmful results.

The first low-phosphorus group, Group A, consisted of five animals and was fed a ration composed of timothy hay, polished rice, corn-gluten meal, and beet pulp or wheat flour. This ration was much lower in its phosphorus content than the ration fed the normal group but otherwise was very similar in its nutritive value.

The second low-phosphorus group, Group B, consisted of four animals and was fed a ration similar to that fed Group A except that wheat gluten was used in place of corn-gluten meal as the principle source of protein for the first fifteen months. After the first fifteen months the animals in Group

B were fed the same ration as were the animals in Group A. The wheat gluten supplied considerably more digestible crude protein in proportion to phosphorus than did the corn-gluten meal. As a result somewhat less phosphorus was supplied in the ration fed the calves in Group B for the first fifteen months of the experiment.

The timothy hay used would grade as U. S. No. 1 and was fed at the rate of about  $1\frac{1}{2}$  pounds per 100 pounds live weight per day. The remainder of the digestible crude protein and total digestible nutrients required to meet the average of the Morrison feeding standard for dairy heifers was supplied by the respective grain rations fed each group.

### *Feed Analysis*

Analyses of all feeds for moisture, ash, protein, ether extract, crude fiber, calcium, and phosphorus were made by the usual methods on composite samples taken when new supplies of feed were purchased. Whenever possible the average analyses were converted to the digestible basis by the digestibility coefficients for American feeding stuffs as given by Henry and Morrison (10); otherwise the digestibility coefficient of some closely-related feed was used.

### *Feeding and Care of Animals*

The calves were fed whole milk until they were about six weeks of age, after which they were gradually changed to skimmilk. The respective grain and hay rations were offered as soon as the calves would consume them. The skimmilk was gradually withdrawn from all groups and the calves transferred entirely to the grain and hay rations at the age of six months. Feed and water was offered twice daily and an accurate account was kept of the amounts consumed. Salt was incorporated in the various grain rations at the rate of 1 pound in each 100 pounds of the grain mixture.

The calves were housed in a well-lighted and ventilated experimental barn but were not exposed to direct sunlight. Each calf was kept in an individual pen of convenient size and fed separately. The animals were confined in their pens continuously except when they were removed to be weighed, measured, and photographed. Wood shavings were used as bedding.

All animals were weighed and the height at withers was measured at weekly intervals. Weights and measurements were made at about the same hour each day in order to eliminate, as much as possible, the difference in weight due to feeding and watering.

### *Methods of Blood Analysis*

Composite samples of whole blood were collected from all animals at monthly intervals according to the method previously described (9).

Trichloroacetic acid filtrates were prepared by adding dropwise, with vigorous stirring, 12.5 mls. of whole blood to 50 mls. of 10 per cent trichloroacetic acid and the mixtures allowed to stand approximately 5 minutes, after which they were filtered through a No. 40 Whitman filter paper. With this amount of whole blood and trichloroacetic acid insufficient filtrate was obtained for triplicate determinations of both calcium and inorganic phosphorus in case the usual duplicate determinations did not check and additional filtrate was desired.

Calcium was determined on 10-ml. aliquots of the trichloroacetic acid filtrate (equivalent to 2 mls of whole blood) according to the method described by Rothwell (21).

Inorganic phosphorus was determined on 5-ml. aliquots of the trichloroacetic acid filtrate according to the method of Fiske and Subbarow (6).

### *Effect of Ration on Blood Composition*

In Table 1 is presented the average growth in weight and height at withers and the average daily consumption of digestible crude protein, total digestible nutrients, calcium, phosphorus and phosphorus consumed per 100-pounds body weight by the animals in the various groups.

Figure 1 shows the average amount of inorganic phosphorus in the blood of the animals in the various groups along with the average daily phosphorus consumption and the amount consumed per 100-pounds body weight of the animals. The average deviation of inorganic phosphorus from the mean for the entire period was .44 mg. per 100 mls. of whole blood for the normal group, .42 mg. for the low phosphorus Group A, and .50 mg. for the low-phosphorus Group B. However, greater deviations than those given occurred in the case of the low-phosphorus groups for the period of 6 to 12 months of age.

As a general rule blood analyses were started at about the second or third months of age. However, in the case of three animals in the normal group and two animals in the low-phosphorus Group B, the blood was not analyzed until after the animals were removed from skim milk at 6 months of age. For the normal group, results of analyses for 5 animals normally fed but not a part of this experiment were included in the average results presented up to 6 months of age. These animals were of the same breed and were confined to the experimental barn just as the animals regularly on this experiment.

In the case of the animals fed the normal-phosphorus ration there was a slight increase in the inorganic phosphorus of the blood from the second to the fourth month, after which it remained fairly constant until about the tenth month was reached. After the tenth month there was a gradual decrease as the animals grew older.

The animals fed the low-phosphorus ration in Group A showed a slight

TABLE 1  
Average growth in weight and height at withers: average daily consumption of digestible protein; total digestible nutrients, calcium and phosphorus

AGE IN PERIODS	BEGINNING OF PERIOD WEIGHT AT lbs.	HEIGHT AT BEGINNING OF PERIOD cms.	DIGEST. PROTEIN lbs.	TOTAL DIGEST. NUTR. lbs.	AVE. DAILY CA. gms.	AVE. DAILY PHOS. gms.	PHOS. PER 100 POUNDS LIVE WEIGHT gms.
Normal Phosphorus Group							
12-24 wks.	175	85.8	.72	3.19	9.0	10.4	4.90
24-36 "	293	97.8	.66	5.17	7.2	13.0	4.09
36-48 "	399	106.1	.95	7.22	10.7	18.2	4.12
48-60 "	520	113.2	1.17	8.80	12.7	23.0	4.11
60-72 "	638	118.7	1.34	9.82	15.0	27.3	4.00
72-84 "	772	123.4	1.48	11.01	17.4	30.1	3.70
84-96 "	887	127.2	1.44	11.50	19.3	30.8	3.37
96-108 "	989	130.3	1.45	11.18	20.6	31.0	3.03
Low Phosphorus Group A							
12-24 wks.	184	85.7	.74	3.73	9.8	8.7	3.87
24-36 "	308	98.5	.73	5.75	7.8	6.4	1.79
36-48 "	446	108.1	1.14	7.63	10.3	8.5	1.73
48-60 "	587	115.6	1.26	8.91	12.7	9.7	1.56
60-72 "	688	119.7	1.36	9.50	14.1	10.6	1.48
72-84 "	781	123.8	1.47	10.38	15.5	11.5	1.42
84-96 "	893	126.6	1.32	10.21	16.9	11.6	1.25
96-108 "	951	129.0	1.30	10.00	16.8	11.3	1.15
Low Phosphorus Group B							
12-24 wks.	178	85.8	.70	3.10	9.1	7.0	3.35
24-36 "	292	96.8	.61	4.84	8.2	4.1	1.32
36-48 "	358	104.8	.84	6.57	10.9	5.9	1.44
48-60 "	479	111.2	.92	6.80	10.8	6.4	1.27
60-72 "	587	116.8	1.06	8.01	13.7	8.5	1.35
72-84 "	724	121.0	1.32	9.09	15.8	10.4	1.36
84-96 "	833	125.0	1.41	10.32	18.0	11.8	1.35
96-108 "	966	127.7	1.48	11.40	20.4	12.9	1.27

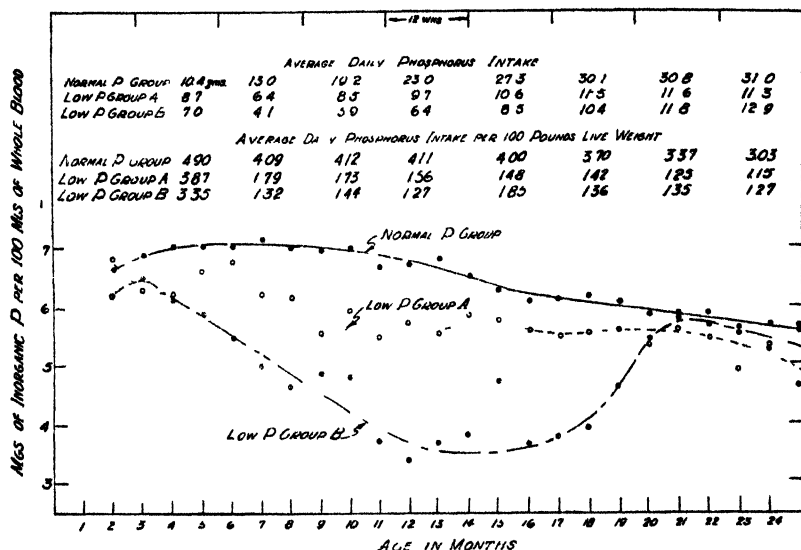


FIG 1 EFFECT OF AGE AND PHOSPHORUS INTAKE ON THE INORGANIC PHOSPHORUS OF WHOLE BLOOD

drop in the inorganic phosphorus of the blood for the third and fourth months, followed by a gradual rise until about the sixth month, after which there was a gradual decline until about the twentieth month was reached. During the twenty-first month the inorganic blood phosphorus rose to about the same level as that of the normal group followed by a gradual decline throughout the remainder of the period.

The animals which were fed the low-phosphorus ration in Group B and which received the least amount of phosphorus for the first 15 months of the experiment, began showing a decline in the inorganic phosphorus of the blood after the third month, which continued until the animals were about 12 months of age. At this time there was present on the average about 3.4 mgr. of inorganic phosphorus per 100-mls. of whole blood, or about half as much as was present in the blood of the animals in the normal group. The inorganic phosphorus continued at a low level until about the eighteenth month, after which there was a gradual increase until at about the twenty-first month it had risen to approximately the same level as that of the normal group. It may be noticed in Figure 1 that Group B received slightly more phosphorus than did Group A during the last two 12-week feeding periods. The inorganic phosphorus of the blood was also slightly higher. This difference was due largely to variations in the consumption of feed by the various animals.

All the heifers on the low-phosphorus ration of Group B showed very definite symptoms of phosphorus deficiency from time to time when the

inorganic phosphorus of the blood was at a low level. However, there was a period of several weeks after the inorganic phosphorus of the blood was reduced below normal, before any physical symptoms were apparent. The exact time varied with the individual animals. The most pronounced physical symptoms of phosphorus deficiency were stiffness in front and rear quarters, unsteadiness in walk, excessive nervousness when excited, and emaciated condition in general. One of these heifers began showing weakness and stiffness in front and rear quarters at about 13 months of age. She went off feed and her condition gradually grew worse until at 14½ months of age she was unable to stand without assistance. It was observed that breathing became heavy and difficult when she was excited or exerted much effort. Often times she would bawl as though in pain. She was slaughtered at the age of 15 months.

At no time did any of the animals in Group A show pronounced physical symptoms of phosphorus deficiency during the period under consideration. It may be seen in Table 1 that there was very little difference in the rate of growth of the animals in the various groups as measured by the rate of gain in body weight and height at withers. However, there was a tendency for the animals on the low phosphorus rations to show signs of loss of appetite, and to make irregular gains, but over the entire period the animals on the low-phosphorus rations made almost as much gain both in body weight and height at withers as did the animals in the normal group.

In Table 2 are presented in summarized form the average amounts of calcium and inorganic phosphorus in the whole blood of the animals in the various groups. Age did not seem to have any appreciable effect on the calcium content of whole blood, whereas there was a definite decrease with age during the second year in the case of the inorganic phosphorus. The level of phosphorus intake did not seem to have any definite effect upon the calcium in whole blood. The averages of the means for the various groups were 7.16 mgs. per 100-mls. of whole blood for the normal group, 7.06 for the low-phosphorus Group A, and 7.62 for the low-phosphorus Group B. When these values are compared there is some indication that the calcium content of the blood of the low-phosphorus Group B was slightly higher than that of the other two groups. Although that might have been the case, the difference could not be considered biologically significant, as the average deviation from the mean for the normal group was .667 mg. of calcium per 100-mls. of whole blood.

#### DISCUSSION

The purpose of this investigation was to study the effect of age and level of phosphorus intake on the calcium and inorganic phosphorus content of the blood in order to determine if possible the minimum phosphorus requirement for growing dairy heifers. Obviously there are a number of other

TABLE 2

*The effect of age and phosphorus intake on the calcium and inorganic phosphorus content of the whole blood*

(Mgs. per 100 mls. of whole blood)

AGE IN MONTHS	NORMAL P. GROUP		LOW P. GROUP A		LOW P. GROUP B	
	Ca.	Inor. P.	Ca.	Inor. P.	Ca.	Inor. P.
2 . . . .		6.67		6.83		6.20
3 . . . .		6.90		6.30		6.50
4 . . . .	7.17	7.03	7.40	6.24	7.20	6.20
5 . . . .	7.03	7.04	6.80	6.62	8.50	5.90
6 . . . .	7.58	7.03	6.74	6.78	7.25	5.50
7 . . . .	7.15	7.18	7.30	6.24	8.25	5.02
8 . . . .	7.20	7.06	6.80	6.16	8.30	4.65
9 . . . .	7.44	7.00	7.58	5.58	8.40	4.90
10 . . . .	7.43	7.01	6.92	5.96	7.90	4.85
11 . . . .	6.94	6.68	6.76	5.52	7.35	3.72
12 . . . .	7.56	6.74	7.34	5.74	7.68	3.43
13 . . . .	7.11	6.83	7.06	5.60	7.38	3.70
14 . . . .	7.42	6.53	7.16	5.90	7.18	3.85
15 . . . .	7.33	6.30	6.58	5.80	7.66	4.73
16 . . . .	6.80	6.15	7.78	5.62	7.50	3.70
17 . . . .	7.50	6.17	7.37	5.54	6.90	3.83
18 . . . .	6.50	6.20	7.35	5.56	6.47	3.97
19 . . . .	7.03	6.10	7.10	5.62	7.85	4.65
20 . . . .	6.90	5.90	7.40	5.36	7.50	5.47
21 . . . .	7.28	5.93	6.70	5.66	7.23	5.90
22 . . . .	6.31	5.93	6.60	5.50	7.93	5.73
23 . . . .	7.75	5.56	6.83	4.95	7.27	5.63
24 . . . .	7.03	5.71	6.65	5.40	8.33	5.30

factors such as the vitamin D content of the ration, season of the year, calcium-phosphorus ratio, and possibly the source of phosphorus in the ration that might affect the level of phosphorus required and the inorganic phosphorus and calcium content of the blood. An attempt was made to control these factors in so far as was practicable.

**Vitamins**—No vitamins were fed other than those occurring normally in the feeds used. The heifers were confined continuously to the experimental barn, which helped to eliminate the effect of sunshine and season. The results of several investigations (11) (22) (3) show that sun-cured hay is a fairly good source of vitamin D. Work at the Michigan Station (11) indicates that sun-cured timothy hay furnishes sufficient vitamin D to protect calves kept out of sunshine from developing rickets. The heifers were fed a good grade of timothy hay at the rate of about 1½ pounds per 100-pounds of live weight. However, with more vitamin D available for calcium and phosphorus utilization the blood of the animals in the low-phosphorus groups might not have been affected to the same degree.

**Calcium-Phosphorus Ratio**—This experiment was not designed to study

the effect of the calcium-phosphorus ratio. However, in the case of the normal group there was always more phosphorus than calcium in the ration with an average calcium-phosphorus ratio of 1 to 1.65. In the case of the low phosphorus groups there was always more calcium than phosphorus in the ration with an average calcium-phosphorus ratio of 1:0.79 for the low phosphorus Group A and 1:0.63 for the low-phosphorus Group B. Theiler, Green, and DuToit (24) concluded that the minimum requirements for growth are higher for phosphorus than for calcium, and that a calcium-phosphorus ratio of 1:4.65 is not necessarily disadvantageous. Meigs and associates (16) experimented with lactating cows and concluded that phosphorus assimilation may be interfered with by an excess of calcium in the ration, and that two parts or more by weight of calcium to one of phosphorus constitutes an excess. However, Gullickson and Eckles (7) did not notice any ill effects with growing heifers fed rations in which the phosphorus content almost invariably exceeded the calcium content by nearly two to one.

### *Phosphorus Requirement*

It may be noted in Table 1 and Figure 1 that the average daily phosphorus consumption for all groups increased as the animals increased in age and more feed was consumed, but decreased slightly in proportion to body weight. Since there was no increase in the amount of phosphorus consumed in proportion to body weight after the eighteenth month, evidently there was a decrease in the requirement for phosphorus in proportion to body weight, as indicated by the rise in the inorganic phosphorus of the blood.

Theiler (23) was of the opinion that the phosphorus requirement was reduced slightly with age. Huffman and associates (12) suggested the possibility that the phosphorus requirement for growth depends largely on the rate of growth rather than on the body weight. They also interpret the results of Hogan and Nierman as indicating that the rate of growth is a good criterion to use in determining the phosphorus requirement for growth.

In Table 3 is presented in twelve-week periods the average number of

TABLE 3  
*Total phosphorus consumption per unit gain in body weight and height at withers*

12 WEEKS PERIOD ENDING WHEN ANIMALS WERE AT AGE OF	NORMAL P. GROUP		LOW P. GROUP A		LOW P. GROUP B	
	Grams of P. per cm. gain in height	Grams of P. per lb. gain in body wt.	Grams of P. per cm. gain in height	Grams of P. per lb. gain in body wt.	Grams of P. per cm. gain in height	Grams of P. per lb. gain in body wt.
24 wks. . . . .	73	7.4	57	5.9	54	5.2
36 " " " " " "	132	10.3	56	3.9	43	5.2
48 " " " " " "	224	13.3	95	5.0	78	4.1
60 " " " " " "	351	16.4	199	8.1	96	4.5
72 " " " " " "	488	17.1	217	9.6	170	5.2
84 " " " " " "	665	22.0	345	8.6	218	8.0
96 " " " " " "	835	25.4	406	16.7	367	7.5

grams of phosphorus consumed per pound gain in body weight, and also per centimeter gain in height at withers between 12 and 96 weeks of age.

It may be noted that there was a fairly consistent increase in the amount of phosphorus consumed per centimeter gain in height at withers by the animals in all groups. There was also a consistent increase in the amount of phosphorus consumed per pound gain in body weight by the animals in the normal group. However, in the case of the animals on low-phosphorus rations the rate of increase of phosphorus consumption per pound gain was much less and very irregular. The relative change in the amounts of phosphorus consumed per centimeter gain in height at withers from 12 to 96 weeks of age was much more than the relative change in the amount of phosphorus consumed per pound gain in body weight. From a study of the changes in the inorganic phosphorus content of the blood, the data indicate that the phosphorus requirement for growth depends to a much greater degree upon the rate of skeletal growth as measured by the rate of gain in height at withers than upon the rate of gain in body weight of the animal. Even though there was a decrease in the amount of phosphorus consumed per 100-pounds body weight as the animals increased in age, nevertheless the amount of phosphorus consumed by the animals in the low-phosphorus groups during the latter part of the experiment was evidently about sufficient to meet their requirements for phosphorus. This resulted in an increase of the inorganic phosphorus in the blood to approximately the same level as that of the normal group.

While there is no definite knowledge as to the optimum amount of phosphorus required by growing dairy animals, several investigators have stated what they believed to be the requirement for such animals, while others have calculated the amounts retained by such animals.

Kellner (13) computed the phosphorus retention of growing calves as 19 grams of  $P_2O_5$  equivalent to 8.3 grams of phosphorus per day for the first year. He was of the opinion that the ration should contain about two or three times this amount. Armsby (2) computed from Lawes and Gilbert's analyses of the ash of the entire body of farm animals that during the first year cattle gained a total of 2972 grams of phosphorus. From this Armsby calculated an average daily retention of 8.14 grams equivalent to 1.7 grams of phosphorus per 100 pounds body weight per animal. This is in close agreement with the results obtained by Kellner (13).

In a study of the mineral requirements of cattle, Theiler, Green, and DuToit (23) found that a ration supplying 2.32 grams of phosphorus and 4.99 grams of calcium per day was deficient. Animals 12 to 18 months of age withstood this level of mineral intake from three to six months after which they showed symptoms of mineral deficiency. When the basal ration was supplemented with 25 grams of bone meal daily, the phosphorus intake was increased to 5 grams per day, and fair growth resulted. When the

bone meal was increased to 100 grams and the phosphorus increased to 13 grams daily, good growth and reproduction resulted. The addition of 2 pounds of wheat bran per day to the basal ration increased the daily phosphorus intake to 11 grams, which resulted in fair gain in body weight. Theiler and associates (25) stated that in any given ration it is the percentage of phosphorus in the ration in relation to the total feeding value which is of importance in causing "osteophagia" or bone chewing of cattle. They found that for cattle of approximately 1000 pounds live weight, a daily consumption of about 11.79 grams of phosphorus per 1000 pounds body weight was somewhere near the point at which osteophagia could develop or disappear. Huffman and associates (12) obtained data which indicated that 10 grams of phosphorus per day furnishes sufficient phosphorus to first calving, especially when calves are fed whole milk for two months, five pounds of sun-cured alfalfa hay per day, and turned into sunshine daily. An intake of 10 to 12 grams per day seemed to be about sufficient to support maintenance, normal growth, and gestation from 18 months of age to first calving.

Since the composition of the blood is a good index as to the severity of phosphorus deficiency, if we assume that the lowering of the inorganic phosphorus in the blood is in direct proportion to the deficiency of phosphorus intake in the feed, the minimum phosphorus consumption required to maintain a normal supply of inorganic phosphorus in the blood under the conditions of this experiment may be calculated. If it is considered that, when the concentration of inorganic phosphorus in the blood is at a normal level, the consumption is sufficient to meet the requirement of the animal, the calculated values would be equal to the minimum requirement for phosphorus.

The calculated values for the average daily requirement under the conditions of this experiment in 12-week periods from 24 to 108 weeks of age are presented in Figure 2. The calculated values for the average daily requirement per 100-pounds body weight were obtained by dividing the amount required per day by the average body weight of all the animals on experiment for the corresponding period. The regression equations were obtained by the method of "least squares," using the calculated values for the average daily requirement, and the average daily requirement per 100-pounds body weight. In the equations "y" represents the requirement in grams per day and "X" the age of the animals in months. The regression lines were plotted according to the regression equation for the average daily phosphorus requirements and the average daily phosphorus requirements per 100-pounds body weight.

It is to be admitted that the severity of phosphorus deficiency as indicated by the lowering of inorganic phosphorus in the blood may not be directly proportional to the deficiency of the phosphorus intake in the feed,

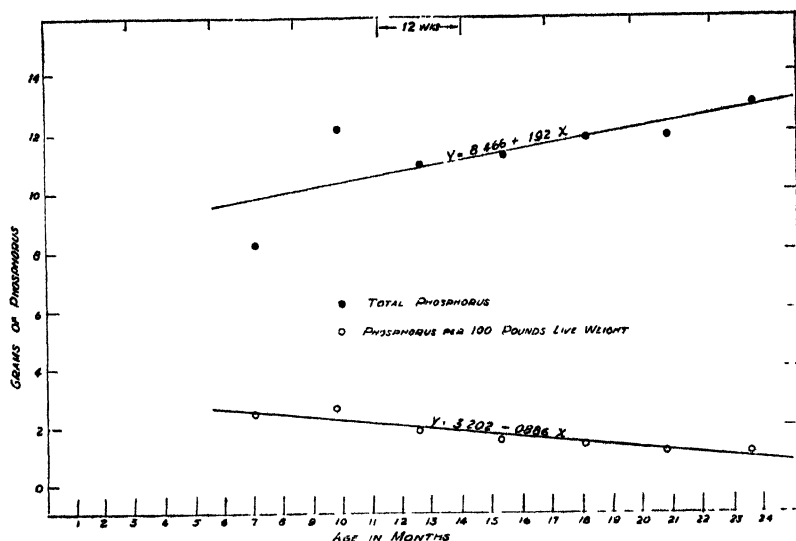


FIG. 2. CALCULATED AVERAGE DAILY MINIMUM PHOSPHORUS REQUIREMENT FOR GROWING DAIRY HEIFERS

especially during the first 48 weeks of age, when the animals were being depleted of their phosphorus reserve. It is commonly known that such factors as age, ration, vitamins, and level of phosphorus intake effect the efficiency of phosphorus absorption and storage. From balance studies it has been demonstrated that phosphorus is used more efficiently at lower levels of intake than at higher levels. There are also differences in the rate at which the inorganic phosphorus in the blood is decreased with lowered phosphorus consumption. The rate at which the composition of the blood is lowered is roughly in proportion to the level of phosphorus intake as may be seen in Figure 1, although the individuality of the animal is an important factor.

For an approximation it is interesting to note that the calculated requirements according to the regression equations are of the same order of magnitude as the values for minimum requirements of growing dairy animals obtained by other investigators. The calculated values thus obtained are slightly higher than an average of 10 grams per day as obtained by Huffman and associates (12) for heifers to first calving. The animals in this experiment were fed timothy hay and confined to the barn continuously. Probably with more vitamin D and sunshine less phosphorus would be required to maintain the inorganic phosphorus of the blood at the normal level.

The requirement for phosphorus was not calculated for the period between 3 and 6 months of age. There was a difference in the trend shown

by the inorganic phosphorus in the blood of the animals in the low-phosphorus Groups A and B during this period, although the average composition of the blood of the two groups was practically the same. When the average composition of the blood of both low-phosphorus groups was compared with that of the normal group there was a difference of .82 mgs. per 100 mls., indicating a slight deficiency of phosphorus for the animals in the low-phosphorus groups. The average daily phosphorus consumption of the animals in the low-phosphorus groups was 7.9 grams and for the animals in the normal group, 10.4 grams. The requirement for phosphorus during the period evidently falls between these two values. If the regression line be extrapolated back to include this period or the requirement calculated according to the regression equations, the calculated average daily requirement would be 9.3 grams for the period of 3 to 6 months of age. Huffman and associates (12) found that 10.3 grams of phosphorus per day appeared to furnish sufficient phosphorus for heifers from 3 to 6 months of age under the conditions of their experiment.

TABLE 4  
*The minimum phosphorus requirements for normal growth of dairy heifers*

AGE IN MONTHS	*ESTIMATED P. REQUIREMENTS (GRAMS)	CALC. P. REQUIREMENT (GRAMS)	CALC. P. REQUIREMENT PER 100 LBS. BODY WT. (GRAMS)
1	6.94	8.7	3.11
2	8.19	8.9	3.02
3	7.74	9.0	2.94
4	11.37	9.2	2.85
5	12.58	9.4	2.76
6	12.08	9.6	2.67
7	10.60	9.8	2.58
8	10.10	10.0	2.49
9	11.39	10.2	2.40
10	10.44	10.4	2.32
11	9.24	10.6	2.23
12	9.68	10.8	2.14
13	7.20	11.0	2.05
14	8.03	11.2	1.96
15	7.47	11.4	1.87
16	11.05	11.5	1.78
17	8.05	11.7	1.70
18	10.01	11.9	1.61
19		12.1	1.52
20		12.3	1.43
21		12.5	1.35
22		12.7	1.26
23		12.9	1.17
24		13.1	1.09

\* Estimated by Huffman and Associates (12).

Huffman and coworkers (12) have estimated the phosphorus requirements for normal growth to 18 months of age, based on Eckles normals and Wilson's requirements for maintenance and growth.

In Table 4 is presented the phosphorus requirements for normal growth as estimated by Huffman and associates (12) along with the calculated values from this experiment according to the regression equations. For comparison values have been calculated for the ages from 1 to 6 months. However, the experimental data used in developing the equations did not cover this period.

#### SUMMARY AND CONCLUSIONS

Three groups of growing dairy heifers were fed in such a way that all received approximately the same amount of digestible crude protein and total digestible nutrients in proportion to body weight. One of the groups was fed a normal ration while the others were fed rations low in phosphorus. Calcium and inorganic phosphorus was determined on composite samples of whole blood at monthly intervals throughout the experiment.

Under the conditions of the experiment as outlined the following conclusions were reached:

1. The inorganic phosphorus in the blood of animals fed a normal phosphorus ration shows a slight increase from the second to the fourth month, after which it remains fairly constant until the tenth month, when there is a gradual decline as the animals grow older.

2. The concentration of inorganic phosphorus in the blood is an important index of the severity of phosphorus deficiency in the ration. Low-phosphorus rations cause an immediate lowering of the inorganic phosphorus in the blood. The rate of lowering is roughly proportional to the severity of phosphorus deficiency in the ration. There is always a lowering of the inorganic phosphorus in the blood for a period of several weeks before any pronounced physical symptoms of a deficiency are apparent.

3. Anorexia, or loss of appetite, stiffness in front and rear quarters, and general emaciated condition are physical symptoms which usually follow a lowering of the inorganic phosphorus in the blood when animals are on phosphorus deficient rations.

4. The phosphorus requirement for growing dairy animals is not directly proportional to gain in body weight, but depends to a considerable degree upon the rate of skeletal growth as measured by the rate of gain in height at withers. There is a decrease in the requirement for growth in proportion to body weight with increase in age as the animals approach maturity.

5. An average daily phosphorus intake of approximately 25 grams (equivalent to about 3.8 grams per 100-pounds body weight) was sufficient to maintain what appeared to be a normal supply of inorganic phosphorus in the blood up to 25 months of age. An average daily phosphorus intake

of 8 grams (equivalent to 1.3 grams per 100-pounds body weight) was not sufficient to maintain a normal supply of inorganic phosphorus in the blood of growing dairy animals from 6 to 25 months of age.

6. Age and level of phosphorus intake were without effect upon the calcium content of whole blood of growing dairy heifers.

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## THE VITAMIN A CONTENT OF PASTURE PLANTS

### III. ALFALFA (*MEDICAGO SATIVA* L.) AND SMOOTH BROME (*BROMUS INERMIS* LEYSS.) UNDER PASTURAGE CONDITIONS AND FED GREEN\*

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This paper is the third of a series from this station on the vitamin A content of pasture plants under pasturage conditions and fed green. Plants previously reported on are: white clover, Kentucky blue grass, timothy, and red top. In the first two papers the reasons for interest in the vitamin A content of pasture plants were discussed and representative literature on the subject was reviewed. Citations will be limited to literature pertinent to this report.

The two plants upon which the vitamin A content is herein reported are alfalfa and smooth brome grass. All the alfalfa and part of the brome were grown under irrigation at the Caldwell Substation of the Idaho Agricultural Experiment Station. Two groups of rats were fed brome grown without irrigation at Moscow. The alfalfa used was the Grimm variety.

#### EXPERIMENTAL PROCEDURE

Essentially the same procedure was used as in the two previous reports (3, 4). Vitamin D was supplied by three drops of Mead's Viosterol per week, as in the second report. Groups I to IV inclusive were fed alcohol-extracted casein as a vitamin A free protein source as in the first investigation (3) while groups V to VIII were fed heat-treated casein as reported in the second paper (4). Fresh green samples of alfalfa and brome were received twice a week. The samples of brome consisted mostly of medium sized leaves with very few stems. The alfalfa samples were leafy, but due to the rankness of growth considerable quantities of stems were present. Pasturage of each plant was typical of ordinary conditions. The experimental feeding period was April 18 to June 15 in 1932 and April 17 to June 12 in 1933.

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## RESULTS

The average weight of the rats in all groups was 41 grams at the beginning of the depletion period and 110 grams at the end. The depletion period for all the rats averaged 31 days.

Table 1 and Figure 1 show the average growth response of the rats fed each of the pasture plants. Results are presented for periods of four weeks (28 days), and the rat units per gram are calculated from the daily dose in milligrams required to cause a gain of 12 grams in 4 weeks. All rats which had not attained a minimum gain of 12 grams in the 4-week period were excluded from the results for average performance as required for assaying U. S. P. standard cod liver oil (1). The rats thus excluded from the averages are indicated by the difference between "rats fed" and "rats used" in Table 1.

When 60 milligrams of alfalfa were fed, the average total gain per rat (9 rats) was  $32 \pm 2$  grams, or 8 grams per week, which would indicate  $269 \pm 17$  rat units of vitamin A activity per gram. Rats fed 180 milligrams per week made an average total gain of  $55 \pm 5$  grams in four weeks.

In 1932 eight rats, litter mates of those receiving 60 milligrams of alfalfa, made an average total gain of  $47 \pm 3$  grams in four weeks when fed 60 milligrams of brome grass from Caldwell. This would indicate a vitamin A value of  $396 \pm 27$  units per gram as compared with  $269 \pm 17$  for alfalfa. Similarly, 11 animals fed 180 milligrams per week made an average total gain of 63 grams. In 1933 additional tests with brome grass were made. Three rats receiving 60 milligrams of this grass from the substation at Caldwell made an average gain of 44 grams which was in agreement with the previous year's results with grass from the same source. In the same year brome grass grown at Moscow (non-irrigated) gave an average total gain of  $34 \pm 3$  grams for eight rats when fed at a level of 60 milligrams per week.

The coefficient of variation in the group fed alfalfa (60 mg. per week) was 26 per cent, while it was 27 in the group fed the same level of brome grass, from the same source (Caldwell). The variation in both groups was less than that reported by Sherman and Burtis (2).

Statistical analysis of the results showed that a true difference existed between the Caldwell brome and the Moscow brome when each was fed at the rate of 60 milligrams per week. The difference was 114 rat units and the probable error of the difference was 26. Since three times the probable error of the difference ( $3 \times 26 = 74$ ) was not equal to the difference (114), some difference not due to methods was obtained. This difference in favor of the Caldwell brome may have been due to loss of moisture in shipping, thereby resulting in a more concentrated product than in the case of the Moscow brome which was taken directly from the field to the laboratory.

Comparison of results obtained from Caldwell brome and Caldwell alfalfa, each fed at a level of 60 milligrams, showed a difference of 127 rat units in favor of brome. The probable error of the difference was 22 units.

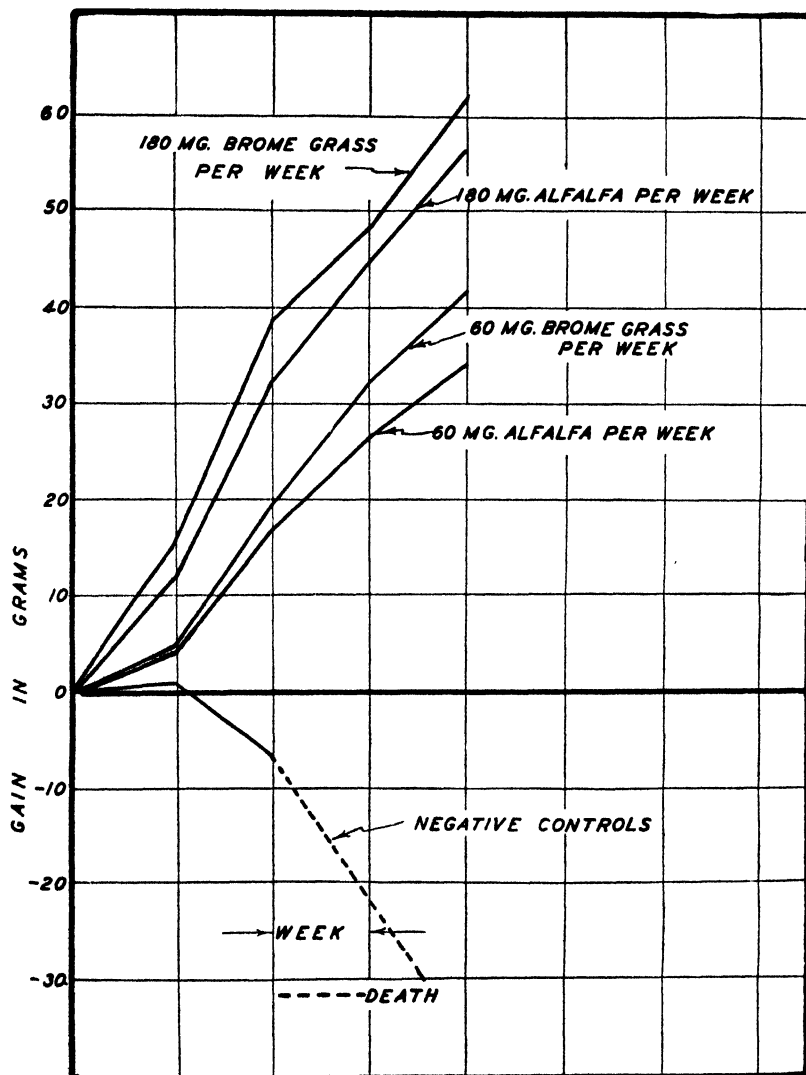


FIG. 1. Average weekly weight increases of rats, previously depleted of vitamin A, when fed either alfalfa or smooth brome as a supplement to a vitamin A free diet.

Average weekly losses in weight of negative control rats were computed each week by averaging the weight of all living rats as long as 50 per cent of the original group survived. The line was terminated by extending it directly from the last week showing 50 per cent survival to a point indicating the average survival days and average weight at death for the entire group. The broken line indicates that one or more deaths occurred during the week.

TABLE 1  
Average growth response of rats when fed fresh alfalfa and brome grass

PASTURE PLANT	SOURCE	DATE OF TEST PERIOD	AMT. OF PLANT FED WEEKLY	RATS FED	RATS USED		AVE. WT. AT BEGINNING OF TEST PERIOD	AVE. WT. AT END OF 4 WEEKS TEST PERIOD	AVE. GAIN IN WT. IN 4 WEEKS	CORRECTION OF VARIATION	RAT UNITS OF VITAMIN A
					No.	%					
Alfalfa	Caldwell	4-18-32 to 6-15-32	0.060	10	9	90	116	148	32 ± 1.9	26	269 ± 17
Alfalfa	Caldwell	4-18-32 to 6-15-32	0.180	10	9	90	117	173	55 ± 4.8	36	154 ± 14
Brome Grass	Caldwell	4-18-32 to 6-15-32	0.060	8	8	100	116	163	47 ± 3.2	27	396 ± 27
"	"	"	0.180	11	11	100	117	180	62 ± 4.4	34	173 ± 12
"	"	4-17-33 to 6-12-33	0.060	3	3	100	104	148	44	*	*
"	Moscow	4-17-33 to 6-9-33	0.060	8	8	100	100	134	34 ± 3.2	37	282 ± 27
Negative Controls				15			118				
								Ave. survival—25 grams Ave. loss in Wt.—30 grams			

\* Insufficient data.

The difference was a true one, it being equal to more than  $5\frac{1}{2}$  times the probable error. Thus brome grass was superior to alfalfa as a source of vitamin A activity when fed at the same level. Observations of the test animals during the feeding period seemed to substantiate the superiority of brome when fed at the same level as alfalfa.

In order that the results obtained may be compared with data reported on the basis of 24 grams gain in eight weeks, the average gain made by each group in eight weeks is given. When the average gain for the longer period is the basis used for calculating the rat units per gram, all animals in a group are usually included whether or not they are gaining at the end of the test. All animals, therefore, are used in summarizing the eight-week averages.

When fed alfalfa at the rate of 60 milligrams per week, the animals (9 rats) made an average total gain of  $42 \pm 3$  grams in eight weeks, indicating the vitamin A activity to be  $175 \pm 14$  rat units per gram. Brome grass fed at the same rate produced an average total growth of  $65 \pm 5$  grams, or  $271 \pm 20$  rat units of vitamin A activity per gram.

The 15 animals used as negative controls survived an average of 25 days and lost an average of 30 grams in body weight. All rats had typical symptoms of vitamin A deficiency.

#### DISCUSSION

The six pasture plants which have been studied under pasturage conditions would rank as follows in vitamin A activity per gram: Smooth brome,  $396 \pm 27$  rat units; red top,  $308 \pm 10$ ; alfalfa,  $269 \pm 17$ ; white clover,  $242 \pm 19$ ; timothy,  $220 \pm 13$ ; and Kentucky bluegrass,  $175 \pm 11$ . The high ranking of these plants compared with other foods and feeds as sources of vitamin A activity should again be emphasized.

It is difficult to attach any significance to the type of plant and its content of vitamin A activity. Alfalfa and white clover are legumes, and the other four plants are non-legumes. Both the highest and lowest ranking grasses are non-legumes. Other characteristics such as rankness of growth, size of leaf, depth of root, etc., do not appear to have any relationship to vitamin A activity of the plant.

Although no tests were conducted, it is probable that significant differences do exist in the carotene content of the various plants, which may in turn harmonize with the rank of the plants in content of vitamin A activity.

All the plants were assayed for vitamin A activity in the early spring and samples were taken from fields being pastured by dairy cows. Undoubtedly different results would be obtained if the plants had been allowed to become more mature without pasturage, as the plants differ considerably in the degree of green color at maturity. From the standpoint of the effect on

the vitamin A activity of butter it is important to study the plants under the same conditions as they are consumed by the cow in grazing.

#### CONCLUSIONS

The results indicate that when sampled under pasturage conditions Smooth brome grass (*Bromus inermis* L.) contained  $396 \pm 27$  rat units of vitamin A activity per gram, and alfalfa (*Medicago sativa* L.) contained  $269 \pm 17$  units. A rat unit as used in this paper is calculated from the daily dose in milligrams required to cause an average total gain of 12 grams in 4 weeks.

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## THE CREATINE TEST FOR ACETYLMETHYLCARBINOL PLUS DIACETYL IN BUTTER CULTURES\*

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The investigations of van Niel, Kluyver and Derx (8) and of Schmalfuss and Barthmeyer (7) established the importance of diacetyl as an aroma constituent of butter and suggested that the diacetyl is formed through the oxidation of acetylmethylcarbinol. Following these studies, the Iowa Agricultural Experiment Station (4) reported data showing that butter cultures having a satisfactory flavor and aroma contain relatively large amounts of acetylmethylcarbinol plus diacetyl, while butter cultures lacking in flavor and aroma contain comparatively small amounts or none of these materials.

The quantitative determination of acetylmethylcarbinol plus diacetyl in a butter culture usually involves the steam distillation of a portion of the culture, after adding ferric chloride to oxidize the acetylmethylcarbinol to diacetyl, and the precipitation of the distilled diacetyl (both that originally present and that formed from the acetylmethylcarbinol) as nickel dimethylglyoximate, which is filtered off and weighed. This procedure is time-consuming and can be carried out only with considerable laboratory equipment. A simple and rapid test which would permit an approximation of the amount of acetylmethylcarbinol plus diacetyl in a butter culture would be useful in comparing cultures from day to day and also in checking the cultures used in a plant or in a creamery organization.

In an investigation of the nature of the Voges-Proskauer reaction, Harden (1) showed that acetylmethylcarbinol, produced from glucose by certain organisms, is one of the necessary factors in the reaction and that some substance present in peptone water is also required. Harden and Norris (2), working with diacetyl, found that the reaction is dependent on the presence of some material, such as arginine or creatine, containing a certain atomic configuration. The importance of acetylmethylcarbinol in the Voges-Proskauer reaction and its formation by various organisms have centered attention on it as a product of bacterial metabolism, and many methods have been suggested for its rapid detection in bacterial cultures. O'Meara (5) considered these methods generally unsatisfactory and proposed growing the organism under investigation in a special medium and then testing for acetylmethylcarbinol by adding creatine and strong sodium hydroxide, shaking, allowing the mixture to stand, and examining for the appearance of a red color.

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The method which O'Meara employed seemed to be applicable to the rapid testing of butter cultures for acetylmethylcarbinol plus diacetyl and, accordingly, was tried for this purpose. The results obtained indicate that the method can be used to advantage in the examination of butter cultures.

The general procedure employed in the rapid examination of a butter culture for acetylmethylcarbinol plus diacetyl is as follows: Two and one-half cc. of the culture are poured into a test tube having an inside diameter of about 0.5 inch and a small amount of creatine (a portion of the powder equal to about one-third of the volume of a wheat grain) added; the creatine can best be handled on the point of a small knife. Strong sodium hydroxide (40 gm. made up to 100 cc. with water) equal in volume to the volume of culture is then poured into the tube, the mixture thoroughly shaken and allowed to stand. If relatively large amounts of acetylmethylcarbinol plus diacetyl are present in the culture, a red color develops at the surface of the mixture in a few minutes or less and soon extends to a depth of 0.25 inch or more. With smaller amounts of acetylmethylcarbinol plus diacetyl the red color develops more slowly and the colored portion is comparatively shallow. Cultures which are decidedly lacking in flavor and aroma may show no red color even after holding the test mixture for hours. When a strong reaction is secured, the material adhering to the wall of the tube above the surface of the liquid is strikingly red in color.

If a series of butter cultures is arranged, by a competent judge, in the order of the intensities of the desirable flavors and aromas and then tested with creatine and sodium hydroxide, the intensities are closely correlated with the rapidities and extents of the color reactions. The butter cultures in use in plants show all degrees of color development with the test and the results of the test are, in general, in agreement with the intensities of the flavors and aromas, as determined by a competent judge. Occasionally butter cultures are seen which show no red color with the test, and these are conspicuously lacking in flavor and aroma. Milk cultures of *Streptococcus lactis* commonly fail to show any red color. In general, more red color is secured with butter cultures made from milk to which citric acid has been added than with cultures made from unmodified milk.

The formation of only a thin red band on the surface of the test mixture should not be interpreted as indicating that the flavor and aroma of the culture are entirely satisfactory, since this may occur with cultures which yield only a relatively small amount of nickel dimethylglyoximate when the acetylmethylcarbinol plus diacetyl is determined quantitatively. A culture with a high flavor and aroma rapidly gives a rather broad band of red color.

The creatine test makes it possible to quickly secure general information on the comparative amounts of acetylmethylcarbinol plus diacetyl present in butter cultures. For the experienced judge such information is useful as a means of confirming the impressions obtained through the senses of taste

and smell, while for the inexperienced judge it can be used to help in developing the ability to properly evaluate cultures. When the method is to be employed in a plant or creamery organization, it should be standardized as well as possible by using (a) approximately the same amount of creatine, (b) sodium hydroxide of a uniform strength, (c) uniform volumes of culture and sodium hydroxide, etc. The importance of approximately the same amount of creatine in various tests is suggested by the fact that Walpole (9) utilized the color given in alkaline solution by creatine when a trace of diacetyl is added for the quantitative estimation of creatine in urine. Levine, Epstein, and Vaughn (3) combined creatine and potassium hydroxide as one solution (0.3 per cent creatine in 40 per cent potassium hydroxide) for use in the Voges-Proskauer test; the solution was found to have limited keeping qualities, and these were greatly influenced by the temperature of holding. In the studies on butter cultures a solution of 0.3 per cent creatine in 40 per cent sodium hydroxide (40 gm. made up to 100 cc. with water) was tried, but the solution seemed to keep poorly at summer temperatures. The mechanism of the Voges-Proskauer reaction and the diacetyl reaction for proteins has been studied recently by O'Meara (6).

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## A COMPARISON OF METHODS OF DETECTING STREPTOCOCCI IN FRESHLY DRAWN MILK SAMPLES

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Recent investigations on the etiology of infectious bovine mastitis emphasize the importance of detecting streptococci in the diagnosis of the disease. Hucker (4) found that milk from a normal quarter free from fibrosis does not contain long-chained streptococci. Ernst, Schmidt-Hoensdorf and Schmidt (1) succeeded in producing typical streptococcal mastitis by introducing streptococci from mastitis milk into the udder by way of the teat canal. Minett, Stableforth and Edwards (8) and Plastridge, Anderson, White and Rettger (10) have shown that herds free from infectious streptococcal mastitis may be established by the use of periodic bacteriological tests and segregation of animals shedding streptococci of the type associated with infectious bovine mastitis. While other organisms, particularly staphylococci, are occasionally responsible for udder disturbances, the evidence at hand indicates that a fairly well defined species of streptococcus is the principal etiological agent to be considered in attempts to control infectious bovine mastitis.

The recognition of streptococci as the principal cause of infectious chronic mastitis increases the importance of methods of detecting the presence of these organisms in the udder of the cow. The standard nutrient agar plate commonly used in determining the bacterial content of milk is generally recognized as unsatisfactory for the detection of udder streptococci. As blood agar prepared with beef or veal infusion supplies the growth requirements of most streptococci and at the same time yields information on the characteristics of the strains isolated, blood agar has been used widely in detecting streptococci in milk samples. Three principal methods of inoculating blood agar plates have been used: namely, (1) streaking one or several loopfuls of whole milk over the surface of the medium, (2) streaking sediment from centrifuged portions of the sample over the surface of the medium, and (3) plating diluted portions of the sample in blood agar.

Microscopic examination of stained films of fresh samples and films prepared from sediment have also been widely used. While the finding of long chains of streptococci by these two methods is generally regarded as conclusive evidence of streptococcal mastitis, the work of Rosell (11), Hucker (5), Halverson, Cherrington and Hansen (3) and Minett, Stableforth and Edwards (7) and Edwards (2) shows that animals affected with chronic

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mastitis commonly fail to shed a sufficient number of streptococci to be detected by these methods.

Recently, particular attention has been given to the significance of the finding of long chains of cocci in incubated samples. Halverson, Cherrington and Hansen (3) regard the mere presence of long chains of streptococci in incubated samples insufficient evidence of streptococcal mastitis, unless correlated with other findings. Hucker, Trudell and Jennings (6) consider the microscopic examination of incubated milk the most effective method of detecting udder streptococci.

Studies on infectious bovine mastitis have been in progress at the Storrs Agricultural Experiment Station since 1926. During the past two years emphasis has been placed on methods of diagnosing infectious mastitis. As a part of this investigation, six different methods of detecting the presence of streptococci in freshly drawn milk samples were compared for the purpose of selecting the most efficient means of detecting shedders of streptococci which are capable of causing infectious bovine mastitis. A detailed account of the results of other tests employed in the diagnosis of mastitis will appear in a later publication.

#### METHODS

Various tests for evidence of mastitis were made on the animals in the Connecticut State College herd at intervals of two weeks over a period of several years. In two of the regular tests, six different methods of detecting streptococci were employed, in addition to the determination of the leucocyte count, sediment volume, reaction to the bromthymol blue test, and the appearance of the sample. The results given by the different methods, and the relation of the results to each other were considered with other evidence of mastitis before, at the time of, and after the tests were made.

*Collection of Samples.*—Before collecting the samples the lower portion of the udder and teats, including the opening of the teat canal, were washed with a solution containing 500 parts of chlorine per million parts of water. Badly soiled udders were given a preliminary washing with water. After discarding the first four streams, separate samples were collected from each quarter of the experimental animals.

*Examination of Samples.*—A detailed account of the methods used in the examination of the samples, and the basis of classifying animals as negative, suspicious or positive for mastitis, have already been reported in Storrs Experiment Station Bulletin 197 (10).

*Detection of Streptococci.*—A brief statement of the methods employed in detection of streptococci follow:

1. A four mm. loopful of the whole milk sample was streaked over the surface of a blood agar plate.

2. A 1 to 10 dilution of the sample was prepared and plated in blood agar, according to the method described in *Standard Methods of Milk Analysis*, Sixth Edition, page 64 (12).

3. A four mm. loopful of the material remaining in the conical end of a centrifuge tube after centrifuging a 10 cc. portion of the sample was streaked over the surface of a blood agar plate.

4. A direct microscopic examination was made of stained films of the fresh samples prepared according to the method of Breed. The presence or absence of cocci occurring in pairs or chains was noted.

5. Films prepared from the sediment obtained from centrifuged portions of the sample were also examined for the presence of streptococci.

6. A microscopic examination was made of stained films prepared from portions of samples which had been incubated from 18 to 20 hours at 37° C. The presence or absence of streptococci occurring in chains of ten or more cells was recorded.

*Evidence of Mastitis.*—Samples were classified as positive or negative for mastitis, on the basis of the appearance of the sample, sediment content, reaction to the bromthymol blue test and the leucocyte count. A detailed description of all possible combinations of results given by the tests employed is considered out of place here. In general, an abnormal appearance of the sample, more than 0.5 per cent by volume of sediment, the yielding of a distinctly abnormal color to the bromthymol blue test and a leucocyte count of over one million cells per cubic centimeter, were all regarded as evidence of mastitis. The results obtained on samples collected during a period of two weeks following parturition and during the last 30 days of the lactation period were not used in this study.

## RESULTS

Three hundred and sixty milk samples were examined for the presence of streptococci by the six methods described.

*Blood Agar.*—The number of samples yielding weakly hemolytic streptococcus-like colonies on blood agar inoculated by the three methods employed is given in Table 1.

As expected, blood agar plates inoculated by streaking a loopful of whole milk over the surface of the medium detected the presence of streptococci in fewer samples than the other two methods. Weakly hemolytic streptococcus-like colonies were found on 51 or 14.2 per cent of the plates streaked with whole milk, on 66 or 18.3 per cent of the plates streaked with sediment and on 62 or 17.2 per cent of the plates inoculated by the standard dilution method.

Preliminary centrifuging of a 10 cc. portion of the sample and the use of a four mm. loopful of the resulting sediment in streaking one-half of the surface of a blood agar plate was more effective in detecting the presence

TABLE 1  
*Detection of Streptococci with the Blood Agar Plate*

METHOD OF INOCULATION	WEEKLY HEMOLYTIC STREPTOCOCCUS LIKE COLONIES					
	Streptococci		Diphtheroids		None	
	number	per cent	number	per cent	number	per cent
Whole milk streaked over surface	51	14.2	0	0.0	309	85.8
Sediment streaked over surface	66	18.3	1	0.3	293	81.4
Dilution*	62	17.2	0	0.0	298	82.8

\* 1 cc. of a 1 to 10 dilution plated in blood agar.

of streptococci than either of the other two methods. For this reason and because the time involved in centrifuging the sample is offset by the saving in materials and glassware, the sediment-streak method of inoculating blood agar plates is considered preferable to the other methods employed in the routine detection of streptococci by means of the blood agar plate. However, if contamination occurs at the time the samples are collected or if the samples are not placed at refrigerator temperature immediately following collection the presence of colonies of udder streptococci is more readily obscured by contaminating organisms on the sediment-streak plates than in the plates inoculated by the dilution method.

TABLE 2  
*Detection of Streptococci by Microscopic Examination*

MATERIAL EXAMINED	STREPTOCOCCI (CHAINS OF 10 OR MORE CELLS)		COCCI OCCURRING IN PAIRS OR CHAINS OF LESS THAN 10 CELLS		NEITHER PAIRS NOR CHAINS OF COCCI	
	number	per cent	number	per cent	number	per cent
Stained films prepared from fresh samples	0	0.0	3	0.8	357	99.2
Stained films prepared from sediment	0	0.5	5	1.4	353	98.1
Stained films prepared from incubated milk	116	32.2	3	0.8	241	67.0

*Microscopic Examination.*—The results obtained by direct microscopic examination of films prepared from the fresh samples, films prepared from the sediment obtained from a centrifuged 10 cc. portion of the sample, and films prepared from the incubated sample are recorded in Table 2.

Microscopic examination of the 360 samples before incubation failed to show the presence of chains of streptococci in a single instance. Cocci occurring in pairs were observed in films prepared from three samples or only in 0.8 per cent of the total number of samples examined. Culture tests made

on these three samples yielded relatively large numbers of chain-forming streptococci, which were identified as the type most commonly responsible for chronic mastitis. Observations on many samples in addition to those included in this investigation show that chains of streptococci are rarely seen in films prepared from samples freshly drawn from udders affected with persistent chronic mastitis. However, streptococci were commonly seen in films prepared from samples collected from udders affected with an acute attack of mastitis. Although several of the 360 samples used in this investigation were abnormal in appearance, none of the animals from which the samples were obtained showed evidence of acute mastitis.

Microscopic examination of the sediment was but slightly more effective in detecting streptococci than examination of the films prepared from fresh samples. Streptococci occurring in chains were seen in the sediment from two samples, or in 0.5 per cent, and cocci occurring in pairs were seen in five instances or 1.4 per cent of the milk samples examined.

Microscopic examination of the incubated samples revealed the presence of streptococci in a surprisingly large number of the samples. Streptococci occurring in chains were found in 116 or 32.2 per cent of the 360 samples examined. Because streptococci were observed in a much smaller number of samples by the use of blood agar plates, it seemed probable that many of the streptococci seen in the incubated samples were saprophytes.

#### RELATION OF THE FINDING OF STREPTOCOCCI BY THE METHODS EMPLOYED TO LABORATORY EVIDENCE OF MASTITIS

In order to determine the significance of the finding of streptococci by the six methods used, the results given by each method were compared with the presence or absence of other laboratory evidence of mastitis 30 days before, at the time, and 30 days after the tests were made for the presence of streptococci. The results obtained are recorded in Table 3.

With few exceptions, the finding of streptococci by either of the three blood agar plate methods was accompanied by other evidence of mastitis. The few samples in which streptococci were detected by direct microscopic examination or by examination of films made from the sediment also gave other evidence of mastitis. The results obtained by examination of the incubated samples were of particular interest, as this method detected streptococci in a greater number of samples which showed other evidence of mastitis than did the other methods employed. Seventy-eight instances of streptococcal mastitis were detected by the microscopic examination of incubated milk samples, as compared with 61 by streaking the surface of blood agar with sediment. However, of the 116 samples in which streptococci were found following incubation, 38 failed to show other evidence of mastitis.

In general, it appears that the more sensitive the method employed for the detection of streptococci, the greater is the proportion of samples in which

TABLE 3  
*Relation of the Finding of Streptococci by the Methods Employed to Other Laboratory Evidence of Mastitis*

METHOD	SAMPLES SHOWING STREPTOCOCCI	LABORATORY EVIDENCE OF MASTITIS DUE TO STREPTOCOCCI		
		30 days before time of test	At time of test	30 days after time of test
Blood agar plate (whole milk) . . . . .	51*	45	48	47
Blood agar plate (sediment) . . . . .	66*	54	61	58
Blood agar plate (dilution) . . . . .	62*	53	60	58
Direct microscopic examination . . . . .	3	3	3	3
Microscopic examination of sediment . . . . .	7	5	7	6
Microscopic examination of incubated milk . . . . .	116	62	78†	76

\* Colonies resembling those of streptococci responsible for chronic bovine mastitis seen on blood agar plates.

† A total of 79 samples showed evidence of streptococci mastitis. Incubated milk failed to detect the presence of streptococci in one instance, only.

streptococci are found without other evidence of mastitis. Determination of the characteristics of strains of streptococci isolated from incubated milk obtained from quarters showing no other evidence of mastitis at the time of, or shortly after, the culture was obtained, shows that such strains are frequently saprophytes, according to Plastridge, Anderson, Brigham and Spaulding (9).

#### EVIDENCE OF MASTITIS IN QUARTERS YIELDING STREPTOCOCCI IN INCUBATED MILK ONLY

As shown in Table 3, 116 of the 360 samples examined contained streptococci as revealed by microscopic examination of incubated milk, while only 66 yielded colonies resembling streptococci on blood agar (see Table 1); in other words, 50 samples yielding streptococci by microscopic examination of incubated milk failed to yield visible colonies of streptococci on the blood agar plates. In order to gain information on the significance of the presence of streptococci in these samples, other evidence of mastitis at the time of the test was obtained; and the results of other tests made on the quarters involved before and after examination of the fifty samples were considered. The results are summarized in Table 4.

Thirty of the 50 quarters involved showed no evidence of mastitis before, at the time of, or after the finding of streptococci in the incubated samples. Cultures were obtained from these samples in several instances and were

TABLE 4  
*Evidence of Mastitis in Quarters Yielding Streptococci in Incubated Milk Only*

NUMBER OF QUARTERS	STREPTOCOCCI IN INCUBATED MILK	STREPTOCOCCI DETECTED BY BLOOD AGAR PLATES	LABORATORY EVIDENCE OF MASTITIS		
			Two weeks preceding test	At time of test	One month following test
30	+	0	0	0	0
3	+	0	0	0	+
7	+	0	0	+	+
1	+	0	0	+	0
9	+	0	+	+	+

identified as saprophytes. As the quarters from which these samples were obtained failed to shed streptococci on subsequent tests, it seems probable that the streptococci found in the incubated samples originated from outside the udder in spite of the precautions taken against contamination in collecting the samples.

On the other hand, three of the 50 quarters developed other evidence of streptococcal mastitis during the month following the finding of streptococci in the incubated samples. Cultures isolated from these quarters have been identified as the type commonly responsible for infectious chronic mastitis.

Seven quarters which gave no evidence of mastitis two weeks previously gave other evidence of mastitis at the time of the test and also on subsequent tests.

One quarter, only, gave other evidence of mastitis at the time of the test, without showing evidence of mastitis on subsequent tests.

Nine quarters known to have been affected with chronic streptococcal mastitis for some time gave samples in which the causative organism appeared in the incubated milk, but failed to do so on the blood agar plates. Blood agar plates inoculated with portions of samples collected two weeks previously and one month later revealed the presence of streptococci.

While incubated milk occasionally contains streptococci which are not responsible for mastitis, the results presented show that the presence of streptococci in the udder which cause mastitis is detected sooner by the use of incubated samples than by the inoculation of blood agar plates with portions of fresh samples, and that chronic shedders of streptococci are detected more frequently by the use of incubated milk than by the other methods used.

#### RELATIVE EFFICACY OF METHODS OF DETECTING STREPTOCOCCI ASSOCIATED WITH MASTITIS

Of the 360 samples included in this investigation 79 showed positive evidence of mastitis and contained streptococci, as revealed by one or several

of the tests employed; whereas 281 samples failed to show evidence at the time of making the comparisons. In order to determine the relative efficacy of the different methods used in detecting streptococci associated with mastitis, the per cent of the 79 samples yielding streptococci by each method was calculated. The incidence of the appearance on blood agar of colonies of saprophytic streptococci, diplococci or diphtheroids which resembled colonies of streptococci responsible for chronic mastitis is recorded in Table 5 under the heading "false positives." The incidence of streptococci in incubated samples from apparently healthy quarters was determined also. The incidence of false positives was calculated by dividing the number of samples in which the results of a given method indicated falsely the presence of pathogenic streptococci, by the number of samples which showed no other evidence of streptococcal mastitis.

TABLE 5  
*Relative Efficacy of Methods of Detecting Streptococci Associated with Mastitis*

METHODS EMPLOYED	PER CENT OF TESTS WHICH GAVE	
	Positive results	False positives
Blood agar plate streaked with whole milk	60.8	1.1
Blood agar plate streaked with sediment	77.2	1.8
Blood agar plate—dilution method	76.0	0.7
Direct microscopic examination	3.8	0.0
Microscopic examination of sediment	8.9	0.0
Microscopic examination of incubated milk	98.7	13.5

The efficacy of the three methods used in inoculating blood agar plates was as follows: plates streaked with a four mm. loopful of whole milk, 60.8 per cent; plates streaked with a four mm. loopful of sediment, 77.2 per cent; and plates inoculated by the dilution method, 76.0 per cent. While the use of sediment gave a higher percentage of positive findings than the dilution method, plates inoculated with sediment gave a higher percentage of false positives, because of the higher incidence of weakly hemolytic colonies of saprophytic streptococci, diplococci or diphtheroids on plates inoculated with sediment.

On the basis of the results obtained, the finding of streptococci in films prepared from freshly drawn samples or from sediment obtained from a 10 cc. portion of the sample invariably indicated that the sample was obtained from an animal affected with streptococcal mastitis. However, these two methods detected less than 10 per cent of the 79 diseased quarters.

Microscopic examination of incubated milk was 98.7 per cent effective in detecting streptococci in samples collected from quarters affected with streptococcal mastitis. However, streptococci were observed in 38 or 13.5 per cent of the 281 samples collected from quarters which gave no other evidence of mastitis at the time. Three of the 38 quarters developed mastitis during the following month while 35 failed to do so.

#### DISCUSSION

In comparing the relative efficacy of six different methods used for the detection of the causative organism of chronic streptococcal bovine mastitis, 360 individual quarter samples were examined. Seventy-nine or 21.9 per cent of the samples showed laboratory evidence of mastitis and the presence of streptococci by one or more of the tests used. The incidence of positive findings given by the six methods, when used in the examination of the 79 samples collected from affected quarters was as follows:

<i>Method used</i>	<i>Per cent positive</i>
1. Direct microscopic examination of films (whole milk)	3.8
2. Direct microscopic examination of films (sediment)	8.9
3. Blood agar plates streaked with a 4 mm. loopful of whole milk	60.8
4. Blood agar plates inoculated by the standard dilution method (1 cc. of a 1 to 10 dilution)	76.0
5. Blood agar plates streaked with a 4 mm. loopful of sediment from 10 cc. of centrifuged milk	77.2
6. Microscopic examination of films prepared from samples incubated over-night	98.7

The samples in which streptococci were detected by direct microscopic examination of films prepared from whole milk and from sediment were from quarters that had been affected with chronic mastitis for a period of at least a year.

The per cent of the 281 samples from apparently healthy quarters, which gave weakly hemolytic colonies on blood agar that were apparently either saprophytic streptococci, diplococci or diphtheroids was as follows: Blood agar streaked with whole milk, 1.1 per cent, blood agar inoculated by the dilution method, 0.7 per cent, and blood agar streaked with sediment, 1.8 per cent.

Streptococci were detected in films prepared from incubated portions of 38 or 13.5 per cent of the 281 samples which gave no evidence at the time of the test. Three of the 38 quarters yielding these samples gave laboratory evidence of mastitis a month after the test.

#### CONCLUSIONS

Microscopic examination of films prepared from incubated milk revealed the presence of the causative organism of chronic streptococcal bovine mas-

titis in a larger number of instances than (1) direct microscopic examination of films prepared from whole milk or sediment, and (2) blood agar plates inoculated with a 4 mm. loopful of whole milk, or a 4 mm. loopful of sediment, or 1 cc. of a 1 to 10 dilution of the sample.

In the absence of other laboratory evidence of mastitis, the finding of streptococci in incubated samples should not be taken as conclusive evidence of infection with the organism which is commonly responsible for chronic infectious bovine mastitis. The significance of the finding of streptococci in such instances can be determined only by isolation and identification of the streptococcus found in the sample.

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## LESPEDEZA HAY FOR DAIRY CATTLE

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Lespedeza is assuming a rôle of importance in the southern and south-central portions of Illinois, although it has been grown extensively in the southeastern states for a number of years. The crop has a number of uses, namely, for pasture, for seed, for hay, and for soil improvement.

Since lespedeza is a leguminous plant having a protein content similar to that of alfalfa and soybeans and can be grown on soils relatively low in productivity, it gives promise of being a means not only for improvement in the feeding of dairy cattle, but for soil improvement as well.

It was the object of the feeding trial reported herewith to measure the feeding value of lespedeza hay for milk production and for gain in weight of dairy heifers by comparing it with alfalfa hay.

Several determinations of the composition or feeding value of Lespedeza hay have been made. It is assumed that unless otherwise stated in the report that the variety was either *Lespedeza striata* (Japan clover) or *Lespedeza stipulacea* (Korean lespedeza).

Studies of *Lespedeza striata* (Japan clover) hay at the Louisiana Station (1), (2) showed a total protein content of 11.7 per cent, and a digestible protein content of 7.6 per cent. At the early bloom stage, the hay consisted of 55 per cent leaves containing 20 per cent protein and 45 per cent stems containing 8 per cent protein. Exposure of the hay to rain for several days lowered the palatability but changed the chemical composition very little.

Studies of the Korean variety (3), (5) showed that it was lower in protein than alfalfa but (3) about the same as alfalfa in its content of calcium and phosphorus. Five tests in Ohio (9) showed the lespedeza hay to contain 11.4 per cent protein. Two trials (4), (8) with dairy cows indicated that lespedeza hay was slightly less valuable than alfalfa hay while another (7) showed but little difference between alfalfa, lespedeza and soybean hays. In one of these trials (8) the cows yielded less milk but gained more weight during lespedeza hay feeding than when soybean hay was fed. Lespedeza hay fed to steers (11) gave greater gains and greater economy than alfalfa hay or soybean hay.

A study (6) of the composition of *Lespedeza sericea* hay showed it consisted of 52 per cent leaves and fine stems and contained 8.6 per cent protein. Another study (10) of hay of the same variety showed that hay harvested June 4 and 17, July 1 and 15, had protein contents of 12.3 per cent, 11.2 per cent, 10.6 per cent, and 9.5 per cent, respectively. In Ohio (9) sericea hay harvested June 28 to July 14 contained 12 to 13 per cent protein, while

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second cutting hay harvested September 20 from the same plots contained 14.5 to 17.6 per cent protein.

#### DESCRIPTION OF FEEDS USED IN TRIAL

The *lespedeza hay* was of the Korean variety and was grown in Sullivan County, Indiana, a few miles from the Indiana-Illinois state line. The crop was a volunteer stand and very heavy. The field was clipped and raked in July with the result that the hay crop which was harvested in September was nearly free from foreign matter. The crop was cut in early bloom. Part of the hay was cured and baled without exposure to rain. Both the leaves and stems of this part of the hay had a high green color. The remainder of the hay was exposed to rain and both leaves and stems were bleached. Both lots of hay retained the leaves on the stems almost completely. The best hay was fed to cows and the bleached hay to heifers.

The *alfalfa hay* was grown within a few miles of Urbana. That fed to the cows consisted of second cutting hay which was fine stemmed, leafy and had a high green color. The alfalfa hay fed to the heifers was second and third cutting hay somewhat coarser in quality than the other lot, but had a bright green color.

Samples of each of the four lots of hay were sent to the Bureau of Agricultural Economics of the U. S. Department of Agriculture, for grading. The grades assigned were as follows:

Kind of Hay		Grade	Hay fed to
Lespedeza (A)	U. S. No. 1 Extra Leafy Extra Green Lespedeza with 60 per cent leafiness and a trace of foreign material		Cows
Lespedeza (B)	U. S. No. 1 Extra Leafy Lespedeza with 55 per cent leafiness and 5 per cent foreign material		Heifers
Alfalfa (A)	U. S. No. 1 Extra Green Alfalfa with 80 per cent color and 45 per cent leafiness and a trace of foreign material		Cows.
Alfalfa (B)	U. S. No. 1 Alfalfa with 65 per cent color, 40 per cent leafiness and a trace of foreign material		Heifers

The *corn silage* was of good quality. It was made from heavily-eared Reid Yellow Dent corn, well advanced in maturity. It contained about 32 per cent dry matter.

The *grain mixture* was made up according to the following formula: shelled corn, ground, 300 pounds; oats, ground, 320 pounds; wheat bran, 250 pounds; soybean oil meal, 100 pounds; salt, 15 pounds; steamed bone meal, 15 pounds. It contained 88.25 per cent dry matter and approximately 14 per cent total protein.

Dried beet pulp was fed to some of the highest producing cows at the rate of 4.5 pounds per head daily.

## GENERAL PLAN OF FEEDING TRIAL

The trial consisted of two periods, each four weeks in length. Period I was preceded by a 1-week preliminary period and Period II by a 1-week transition period.

Two groups of cows and two groups of heifers were used. Each group of cows consisted of 8 Holsteins and 1 Jersey while the heifer groups contained animals of four breeds. The cow groups were made up by choosing two cows as nearly alike as possible with respect to breed, stage of lactation, milk yield, stage of gestation, etc., and placing one cow of this pair in Group I and the other in Group II. The heifer groups were made up in the same way except that breed, age, size, stage of gestation and amount of feed consumed daily were the factors upon which pairs were selected and division into groups was made.

The animals of a pair were fed exactly the same kind and amounts of feed except for the kind of hay. An attempt was made to have the animals consume very large amounts of hay and to this end silage was fed in small amounts to the cows while the heifers were limited to hay and grain mixture only. The refused portions of the hay were collected from the mangers frequently and placed in gunny sacks. These portions, which absorbed moisture as a result of being nosed over by the cows, were dried and the amounts calculated to the same moisture basis as the hay fed. Samples of all feeds for analysis were taken weekly.

At the close of Period I, the groups of animals which had been fed lespedeza hay were fed alfalfa hay and the groups which had received alfalfa hay were given lespedeza hay.

The animals were weighed on three consecutive days at the beginning and end of each period and also once weekly. Each milking of each cow was weighed and sampled. Composite samples were tested weekly for butter-fat content.

## COMPARATIVE FEEDING VALUES

*Palatability.*—The lespedeza hay was less palatable than the alfalfa hay as determined by a preliminary test that was made to select animals which would consume lespedeza hay in large enough amounts for the trial. Some cows and heifers would not consume lespedeza hay as readily or in as large amounts as alfalfa hay. The fact that alfalfa hay had been fed regularly to the herd prior to this test must be considered, however.

The animals employed in the feeding trial consumed hay in large amounts per head daily and also in relation to body weight, with a negligible amount of hay refused. (Table I.)

*Value for Milk Production.*—The milk yields of the cows during the periods of lespedeza hay feeding were nearly the same as when alfalfa hay was fed. The actual yields were 47.04 pounds daily per cow, while lespedeza

TABLE 1  
*Amounts of Hay Consumed*

KIND OF HAY	KIND OF ANIMALS	AMOUNT FED DAILY PER HEAD	AMOUNT REFUSED DAILY PER HEAD	AMOUNT CONSUMED DAILY	
				Per head	Per 100 lbs. live weight
Lespedeza (A) ...	Cows	lbs. 21.65	lbs. .15 .69	lbs. 21.50	lbs. 1.66
Alfalfa (A) ..	"	21.65	.13 .60	21.52	1.66
Lespedeza (B) ...	Heifers	12.84	.06 .47	12.78	1.72
Alfalfa (B)	"	12.85	.01 .08	12.84	1.72

hay was fed, and 46.55 pounds during the alfalfa hay periods. Because of slight differences in test, a more accurate comparison is obtained by converting the milk yields to an energy basis, as shown in Table 2. While the milk yield and gain in weight were slightly greater during lespedeza hay feeding, these differences are not considered significant.

TABLE 2  
*Summary of Results of Feeding Lespedeza Hay in Comparison with Alfalfa Hay to Dairy Cows*

KIND OF HAY	NO. OF COWS	FEED CONSUMED DAILY PER COW			GAIN IN WEIGHT DAILY PER COW	TEST OF MILK	MILK YIELD DAILY PER COW <sup>1</sup>
		Silage	Hay	Grain			
Lespedeza	18	lbs. 19.3	lbs. 21.5	lbs. 14.9 <sup>2</sup>	lbs. .6	per cent 3.81	lbs. 45.7
Alfalfa	18	19.3	21.5	14.9 <sup>2</sup>	.6	3.68	44.3

<sup>1</sup> Milk energy in terms of 4% milk computed according to the formula  $.4 \times \text{milk (in pounds)} + 15 \times \text{fat (in pounds)}$ .

<sup>2</sup> Includes 2.2 pounds dried beet pulp.

*Value for Gain in Weight.*—Slightly different grades of hay than those fed to the cows were employed in the feeding trial with growing dairy heifers. The gain in weight was about 2 per cent greater during the periods of lespedeza hay feeding than during the feeding of alfalfa hay, but this difference is considered too small to be significant. The gain of approximately 1.3 pounds per head daily is satisfactory for heifers of this weight. No doubt, more rapid gains could have been obtained by reducing the amounts of hay and increasing the amounts of grain fed, but it was considered desirable for the purposes of this trial to have the hay form as large a proportion of the ration as possible.

*Conditioning Effect.*—Careful observations of the laxative properties of the feeds indicated that the rations containing lespedeza hay were no more or no less laxative in their effect than the alfalfa hay rations. No tests to

TABLE 3  
*Summary of Results of Feeding Lespedeza Hay in Comparison with Alfalfa Hay to Dairy Heifers*

KIND OF HAY	NO. OF HEIFERS	LIVE WEIGHT PER HEAD	FEED CONSUMED DAILY PER HEAD		GAIN IN WEIGHT DAILY PER HEAD
			Hay	Grain	
Lespedeza	23	lbs. 745	lbs. 12.78	lbs. 3.61	lbs. 1.32
Alfalfa	23	748	12.84	3.60	1.29

TABLE 4  
*Percentage Composition of Hays Fed*

KIND OF HAY	DRY MATTER	PROTEIN	ASH	FIBER	ETHER EXTRACT	N-FREE EXTRACT	CaO	P <sub>2</sub> O <sub>5</sub>	LEAVES <sup>1</sup>
Lespedeza (A)	84.3	12.7	4.1	19.0	1.9	46.6	3.0	.4	62
Lespedeza (B)	85.9	12.8	4.1	19.1	1.7	48.2	2.8	.4	56
Alfalfa (A)	87.2	16.1	6.6	21.4	2.1	41.0	4.9	.3	49
Alfalfa (B)	88.1	16.3	6.4	26.0	2.3	37.1	4.4	.4	49

<sup>1</sup> Determined by separating large samples into stem and leaf portions.

determine this point were conducted, however. No harmful or undesirable effects upon the health or condition of the cattle which could be traced to the lespedeza hay feeding were observed.

*Chemical Composition.*—The lespedeza hay was found to be lower than the alfalfa hay in its content of protein, fiber, total ash, and lime. Judged by chemical composition alone, the lespedeza hay was inferior in feeding value to the alfalfa hay, although the lower fiber content of the lespedeza hay is a point in its favor. Evidently the hay and grain mixtures of both the lespedeza and alfalfa rations supplied plenty of protein, so that the lower protein content of the lespedeza hay was not a factor which affected the results of the feeding trial.

#### SUMMARY

The feeding value of lespedeza hay was compared with that of alfalfa hay in feeding trials with dairy cows and dairy heifers.

The lespedeza hay was somewhat less palatable than the alfalfa hay.

The particular lot of lespedeza hay fed to dairy cows proved practically equal pound for pound to the alfalfa hay fed, as judged by milk yields and gain in weight of the cows. Slightly lower grades of both lespedeza hay and alfalfa hay were found practically equal pound for pound for gain in weight

of growing dairy heifers. In both trials, however, it is likely that adequate amounts of protein were supplied, so that the lower protein content of the lespedeza hay was not a limiting factor.

No differences in the laxative properties or conditioning effects of lespedeza hay and alfalfa hay were observed.

The lespedeza hay was found to be lower than the alfalfa hay in its content of protein, fiber, total ash, and lime.

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## THE PREPARATION OF MOLD POWDER FOR BLUE-VEINED CHEESES\*

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In the manufacture of blue-veined cheeses the usual method of inoculation has been to sprinkle spores of the desired mold, in the form of a powder, over the curd at the time it is being placed in the hoops. A mold powder is commonly prepared by growing the mold on some medium and then drying and powdering the mass of material. Various methods have been suggested for growing the mold, including (a) the inoculation of baked products specially prepared from rye, whole wheat or barley flour or of the interior of a loaf of ordinary bread (2, 3, 4), and (b) dipping strips of bread into an acid suspension of mold spores (1, 5). These procedures are carried out as aseptically as possible, and the inoculated material is usually incubated at a relatively low temperature. One method of limiting the growth of foreign molds has been to gradually accustom the desired strain of mold to formalin and then dip the bread into an acid suspension of the mold spores containing 1 per cent formalin (5).

In various attempts, at the Iowa Agricultural Experiment Station, to employ the commonly suggested methods for the preparation of mold powder, practical difficulties were encountered which made advisable the use of a method which could be more satisfactorily controlled. These difficulties included (a) the development of foreign molds in the inoculated material, (b) a relatively low yield of powder because of the failure of the mold to grow in a portion of the inoculated material, and (c) the long time required for spores to develop.

### METHOD SUGGESTED

After employing various procedures for preparing mold powder, the following method has been adopted for the preparation of mold powder to be used in the manufacture of Iowa Blue Cheese, a Roquefort type cheese made from cows' milk.

*Medium used.* Whole wheat bread is cut into cubes approximately 0.5 inch on a side and placed in salt-mouth bottles, the bottles being filled from about one-third to about one-half full. The bottles are plugged with cotton and sterilized at about 15 pounds pressure for about 30 minutes, care being taken to raise the temperature very slowly to prevent cracking of the glass. After partial cooling, the bottles are removed from the autoclave and frequently shaken to keep the pieces of bread from adhering in large masses.

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FIG. 1. TYPE OF CONTAINER USED FOR GROWING MOLD.

Left, uninoculated, right, mold well developed.

There seems to be an advantage in sprinkling the unsterilized bread with a small amount of water so that the sterilized material will not be too dry. Sterile water can also be added to the sterilized bread.

*Inoculation.* Two methods of inoculating the sterilized bread have been employed. The first consists of making a suspension of spores in water and adding this to the sterile material. The second and preferred method consists of sterilizing several cubes of bread in a large test tube and inoculating them with the desired mold; after sporulation has occurred the cubes are transferred to the bottles of bread and the bottles thoroughly shaken to distribute the spores. With this procedure a very heavy inoculation of the bread is easily secured.

*Incubation.* The inoculated bottles are incubated at 21° C. until copious spore formation has occurred. The time required varies from 8 to 12 days, depending on the strain of mold being used and, presumably, other factors.

During the incubation the bottles are shaken occasionally to insure heavy inoculation of all pieces of the bread and to keep the pieces from packing together and thus limiting the air supply. Even with frequent shaking, however, the pieces of bread overgrown with mold tend to settle together.

*Drying.* The bread, covered with spores, is removed from the containers and dried in a thin layer on cheesecloth supported by a frame. If desired, cheesecloth can be spread over the layer of bread. The drying is carried out in a warm room and requires from 2 to 4 days. When only a small amount of bread is being dried it can be suspended in a cheesecloth bag.

*Grinding.* After the material is completely dry it is pulverized in a small stone churn to which small stones have been added to serve as balls. Small amounts of bread can be ground in a mortar. The powdered material is passed through a 40-mesh screen to remove the larger particles.

*Storage.* The finished powder is placed in tin cans and held in a cool place until used.

#### GENERAL RESULTS WITH MODIFICATION OF THE METHOD

Various modifications of the method, as outlined, have been used. White bread did not seem to support as good a growth of the molds employed as whole wheat bread. Cracker crumbs were less satisfactory than white bread, probably because the crackers tended to pack into large masses which prevented easy access of air. The addition of an aqueous solution of lactic acid did not appear to improve the growth of the molds with any of the materials used. Large Erlenmeyer or round flasks were employed but seemed to be less satisfactory than the salt-mouth bottles. The larger openings in the bottles may provide a better air supply than the smaller openings in the flasks.

#### SPORE CONTENTS OF POWDERS

The spore contents of the powders that have been made varied from 30 million to 1.5 billion per gram, as determined by the plate method using tomato agar as the medium, with most of the powders having spore contents between 100 million and 300 million per gram. When the spores were suspended in water and a count made with a haemocytometer, much higher values were secured; with this procedure many of the spores were seen to be in groups.

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## A METHOD FOR THE MICROSCOPIC EXAMINATION OF BUTTER

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Many of the organisms of importance in the sanitary and industrial control of butter are not easily detected by routine plating procedures, therefore microscopic examination of butter is advantageous under many circumstances. Hammer and Nelson (1) devised a method which involves the separation of the fatty and non-fatty portions of the butter with the aid of heat and centrifugal force; 0.01 cc. of the non-fatty portion is spread over 1, 4, or 8 square centimeters, stained, and examined by the technique described by Breed (2). The value of such a method for both research and control purposes is obvious.

The following method was designed primarily to eliminate some of the practical difficulties involved in the use of the 0.01 cc. capillary pipette and the centrifuge, and to provide a method which would lend itself to routine examination of large numbers of samples.

The butter to be examined is melted by heating to 45° C. for a period not to exceed 15 minutes, after which it is agitated sufficiently to insure a homogeneous sample.

(a) On a standard 1 x 3 inch. chemically clean, glass slide place 0.1 cc. of the melted butter, 1 drop of xylol, and 1 drop of Mayer's egg-glycerine mixture. To prepare Mayer's solution equal parts of egg albumin and glycerine are mixed, thoroughly beaten, and filtered through cotton. One per cent sodium salicylate should be added as a preservative, and the solution kept in a refrigerator when not in use. Mayer's solution is used to aid in fixing the smear to the slide.

(b) With an "L" shaped platinum needle stir the butter, Mayer's solution, and xylol together until the mixture is opalescent and homogeneous, then spread evenly over the entire area of the slide. It is important that the mixing of the ingredients be properly done before spreading the smear over the area of the slide. Proper mixing requires from 2 to 3 minutes.

(c) Allow the preparation to dry, and at the same time coagulate the albumin by placing the slide on a flat bottle of hot water at approximately 80° C. for 10 to 15 minutes.

(d) Fix the smear in 70 per cent alcohol for 10 to 20 seconds, and allow to dry.

(e) Immerse in xylol 2 minutes, and allow to dry.

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(f) Stain in methylene blue for 1 minute, then wash by dipping the slide once in each of two vessels of water. Allow to dry in the air.

(g) Examine under an oil immersion lens, the field of which has been standardized to a diameter of 0.157 mm. (3). The average number of microorganisms per field multiplied by 1,000,000 gives the number per cubic centimeter of melted butter.

If it is desirable to enumerate objects large enough to be seen with the lower magnifications of the microscope, standardize the diameter of the field of the high dry objective to 0.351 mm. and of the low power objective to 1.57 mm.; multiply the average number of objects observed per field by the factors 200,000 and 10,000 respectively to give the number per cubic centimeter of melted butter. The method described in this paper has been found to be equally advantageous for the microscopic examination of heavy cream.

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## VITAMIN D STUDIES IN CATTLE

### II. THE VITAMIN D SPARING ACTION OF MAGNESIUM IN THE RATION OF DAIRY CATTLE\*

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The possibility of a vitamin D sparing effect of magnesium was noted by Huffman and coworkers (1) in 1930, when Calf C-44 recovered from rickets after commercial magnesium carbonate had been added to the ration. When this supplement was withdrawn from the ration, however, the rachitic symptoms again became pronounced. Several other investigators have made similar observations. von Euler and Rydbom (2) working with rats reported that the addition of magnesium salts to a rachitogenic diet had an antirachitic effect. They explained the results which they obtained upon the basis of increased phosphatase activity due to the magnesium ions. Kay (3) has also demonstrated that magnesium ions stimulate phosphatase activity. Barbour and Winter (4) found a definite calcium retention in dogs which had been fed a basal diet supplemented with magnesium in the form of the gluconate or the lactate. Carswell and Winter (5) also found that magnesium appeared to favor calcium storage in man when the phosphorus intake was adequate. Becka (6) reported that certain magnesium compounds prevented or cured osteomalacia in cattle. Some investigators (7) (8), however, have reported that the addition of magnesium compounds to the diets have resulted in the disturbance of the calcium and phosphorus metabolism. The earlier work on the problem has been reviewed by Elmslie and Steenbock (9).

In a previous report (10), in which the antirachitic value of sun-cured alfalfa was demonstrated, it was suggested that some portion of that value may have been due to the high magnesium content of the hay. The purpose of this investigation was to study the relationship of magnesium to rickets in calves when the basal rachitogenic ration was supplemented with (a) magnesium in the form of the carbonate or the oxide and (b) the efficacy of these compounds when a limited amount of vitamin D was introduced into the ration.

#### EXPERIMENTAL

Twelve healthy grade Holstein calves were used in this work. Calves C-99, C-101, C-104 and C-107 received whole milk up to 45 days of age, after which time the whole milk was replaced with skim milk, corn and oats.

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The corn and oats were replaced at about 90 days of age by a rachitogenic grain mixture, the composition of which appears below :

Yellow corn	50 per cent	Linseed oil meal	8.5 per cent
Oats	20 per cent	Calcium carbonate	1.0 per cent
Corn gluten meal	20 per cent	Sodium chloride	0.5 per cent

The skim milk was discontinued at about 120 days of age. C-99 received 10 cc. of cod liver oil per day and alfalfa hay *ad lib.* to 90 days of age in addition to the regular ration. C-159 had been used in another experiment and was not placed on the basal rachitogenic ration until 296 days of age.

Calves C-170, C-172, C-173, C-174, C-175 and C-176 were fed whole milk up to 30 days of age, after which time they received skim milk, corn and oats, and 5 cc. of cod liver oil per day. The corn and oats were replaced by the rachitogenic grain mixture at 90 days of age but the skim milk was not discontinued until 120 days of age. In addition to the above rations, C-172 and C-175 received one per cent of the dry matter of the ration as  $MgCO_3$  (C.P.) and C-174 and C-176 received an equivalent amount of magnesium as MgO (U.S.P.). The actual magnesium intake of each calf was the same. Calf C-89 received the same basal ration as C-44 (1), namely, a grain mixture and wheat straw.

The calves were kept in the barn during the day but were turned out for exercise at night.

Shavings were used for bedding. The calves were offered water twice daily. Blood samples were taken from the jugular vein at least every two weeks and the plasma analyzed for calcium, inorganic phosphorus (10) and magnesium (11) by methods previously reported. The 8th rib on the right side, a section of the orbital plate and the right dental pad were used for the determination of ash, calcium, phosphorus and magnesium by methods which have already been recorded (10).

#### RESULTS

The results for a representative animal of each group are shown in Tables 1-5 and in Figure 1. The ash contents of the moisture-free, fat-free 8th rib, orbital plate and dental pad are summarized in Table 6.

#### *Effect of Supplementing the Rachitogenic Ration with Magnesium Compounds*

**Calf C-101.** This calf manifested tetany at 95 days of age and again on the 106th day, at which time the calcium and inorganic phosphorus were 6.2 and 8.01 mg. per 100 cc. of plasma. Tetany was again manifested two days later. The concentrations of calcium and inorganic phosphorus during this convulsion were 6.5 and 7.53 mg. Up to this time the calcium, phosphorus and magnesium intakes had averaged 7.0, 7.0 and 1.5 gm. per day respectively. Magnesium carbonate was added to the ration at this time which

TABLE 1

*C-104. Effect of the Rachitogenic Ration, Supplemented with Magnesium Carbonate, Upon the Plasma Calcium, Inorganic Phosphorus and Magnesium*

DATE	AGE	WEIGHT	PLASMA		
	days	lbs.	Ca	P	Mg
mg. per 100 cc.					
1-28-31	90 <sup>1</sup>	176	9.4	6.32	
2-7	100	182	7.6	7.58	
2-17	110	189	7.3	7.27	1.95
2-27	120	197			
3-7	130	207	7.7	6.98	2.10
3-17	140 <sup>2</sup>	211	7.8	5.37	3.47
3-27	150	213			
4-8	160	211	8.2	5.17	2.83
4-18	170	221	7.7	5.93	1.56
4-28	180	230	9.1	5.06	2.66
5-8	190	239	7.4	5.63	2.86
5-12	194	244	7.7	5.14	2.13

<sup>1</sup> MgCO<sub>3</sub> added to ration at 91 days of age.

<sup>2</sup> Legs started to bow.

TABLE 2

*C-89. Effect of a Magnesium Carbonate Supplement Upon the Plasma Calcium and Inorganic Phosphorus When Wheat Straw Furnished the Only Source of Vitamin D*

DATE	AGE	WEIGHT	PLASMA	
	days	lbs.	Ca	P
mg. per 100 cc.				
11-25-30	367	465	10.2	9.33
12-5	377	488	11.4	8.33
12-15	387	491	10.8	8.56
12-25	397	501	10.0	8.47
1-4-31	407	522	8.3	9.40
1-14	417	527	7.6	7.76
1-24	427	536	10.2	8.62
2-3	437	564	6.7	6.90
2-13	447	580	6.8	7.67
2-23	457	581	7.3	8.93
3-2	465 <sup>1</sup>	down	6.3	6.96
3-15	477	"	7.9	6.20
3-25	487	"	9.8	5.78
4-4	497	"	11.0	5.39
4-14	507	"	10.4	5.67
4-21	514	"	9.5	6.28

<sup>1</sup> MgCO<sub>3</sub> added to ration at 464 days of age.

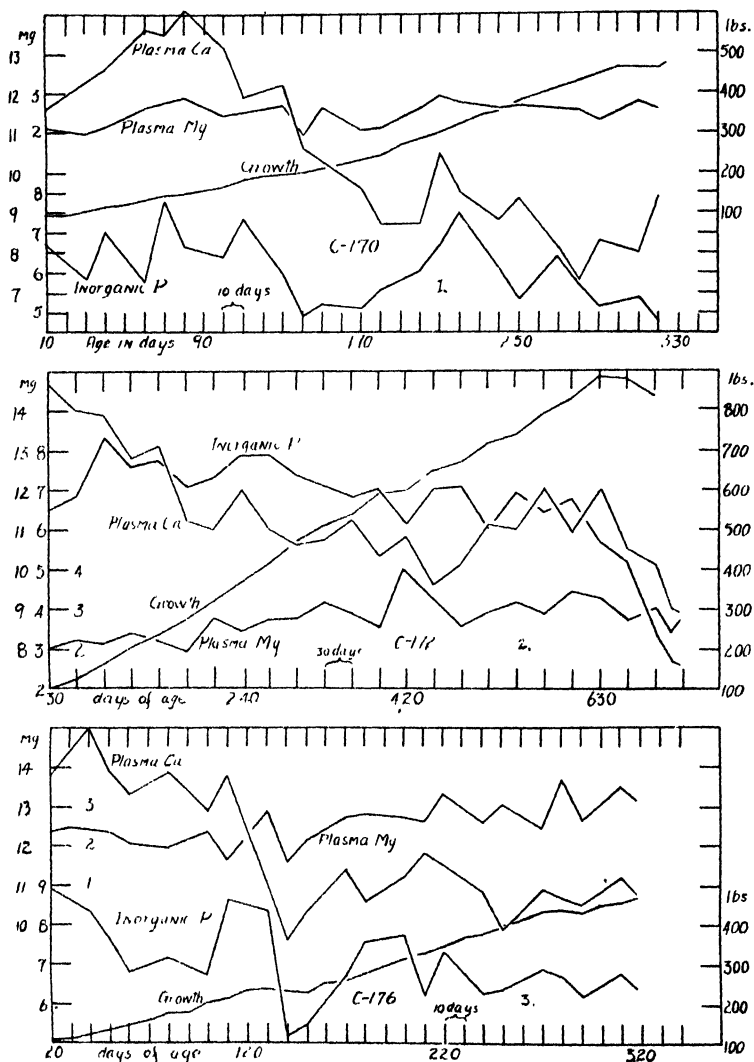


FIG. 1. Plasma calcium, inorganic phosphorus and magnesium curves of calves which received the rachitogenic ration plus 5 cc. of cod liver oil per day, with and without a magnesium supplement.

increased the magnesium intake to 10 gm. per day but the calcium intake decreased because of anorexia. Tetany was not observed again until the calf was 142 days of age. By this time the plasma inorganic phosphorus and magnesium were also much below normal for a calf of this age. The

calf was unable to use its hind quarters following this attack. Two days later it was given 30 cc. of standard cod liver oil and 100 units of parathormone. Another 100 units were given on the following day in an attempt to bring about recovery from coma. On the next day the calf had recovered from the comatose state while the calcium, inorganic phosphorus and magnesium were 5.6, 4.35 and 1.01 mg. per 100 cc. respectively. Although the ration was changed to skim milk and sprouted oats at this time the inorganic phosphorus continued to decline. C-101 died at 176 days of age. A fractured femur was found at autopsy in addition to the severely rachitic bones.

*Calf C-104.* This calf was bled at 90 days of age, at which time the concentrations of calcium and inorganic phosphorus were 9.4 and 6.32 mg. per 100 cc. of plasma. The rachitic grain mixture containing 3 per cent magnesium carbonate replaced the corn and oats at 91 days of age. The change to the high magnesium ration did not aid in maintaining the plasma calcium and phosphorus at normal levels. The first evidence of rickets was observed at 137 days of age but the concentrations of calcium and inorganic phosphorus had already declined to 7.8 and 5.37 mg. The intakes of calcium, phosphorus and magnesium were 7.0, 7.4 and 1.5 gm. per day respectively previous to the addition of the magnesium carbonate, but after the addition, the intakes increased to 15.6, 9.8 and 11.0 gm. per day respectively. When the calf was slaughtered at 194 days of age the plasma calcium, inorganic phosphorus and magnesium values were 7.7, 5.14 and 2.13 mg. respectively. The rib bones were rachitic. Table 1 shows the typical blood findings of the calves in this group, although the inorganic phosphorus declined to much lower levels in some of the calves.

*Calf C-107.* When this calf was first bled at 83 days of age the calcium and inorganic phosphorus were 8.9 and 6.48 mg. per 100 cc. of plasma. The addition of three per cent of the ration as magnesium carbonate at 91 days of age did not prevent the manifestation of rickets at 155 days of age. The concentrations of calcium and inorganic phosphorus had decreased to 8.0 and 3.77 mg. by this time. When the calf was slaughtered at 200 days of age these values were 9.7 and 2.35 mg. Tetany was not observed in this calf.

*Calf C-99.* This calf received the basal ration supplemented with 10 cc. of cod liver oil per day and sun-cured alfalfa hay *ad lib.* to 90 days of age. The blood values were normal at 120 days of age but during the next 60 days the calcium and inorganic phosphorus had steadily dropped to 7.3 and 5.68 mg. per 100 cc. of plasma. Stiffness was not observed until the calf was 205 days of age. The rachitic condition increased in severity, consequently 3 per cent of the rachitic grain mixture was replaced with magnesium carbonate at 234 days of age. Plate I illustrates the physical condition of the calf at this time. The plasma calcium, inorganic phosphorus and magnesium values were 6.3, 5.13 and 1.47 mg. respectively. The ingestion of the magnesium salt failed to relieve the stiffness and caused the appetite to de-

## PLATE I



1. C-173 received the rachitogenic ration plus 5 cc. of cod liver oil per day. Typical rachitic stance. C-174 received the same ration as C-173 supplemented with magnesium oxide.
2. C-172 received the same ration as C-174, no evidence of rickets at 410 days of age.
3. C-174. No evidence of rickets at 404 days of age.
4. C-99. Typical rachitic stance, one rear foot directly behind the other one and straight pasterns. Front legs bowed forward and outward.

crease 50 per cent, but it temporarily increased the plasma calcium to 8.1 mg. and the magnesium to 3.77 mg. There were no significant variations in the inorganic phosphorus. During the development of rickets the calcium, phosphorus and magnesium intakes were 11.5, 7.6 and 4.2 gm. respectively. Tetany was not observed in this calf. C-99 died at 256 days of age

at which time the concentrations of calcium, inorganic phosphorus and magnesium in the plasma were 6.9, 6.83 and 2.53 mg. respectively. The ribs were markedly rachitic.

*Calf C-159.* This calf had been used previously to determine the curative value of viosterol. At 296 days of age the viosterol was discontinued and the calf was placed on the basal rachitic ration. The plasma calcium maintained its normal position until 366 days of age when it abruptly dropped to 8.1 mg. per 100 cc. and progressively declined to a minimum of 6.1 mg. at 395 days of age. Stiffness was also observed at this time. Therefore, sufficient magnesium oxide was added to the ration to increase the magnesium intake from 6.9 to 16.0 gm. per day. In the absence of vitamin D, the addition of the magnesium oxide failed to alleviate the hypocalcemia and did not prevent the rachitic condition from becoming more severe. The calf was removed from this phase of the experiment at 436 days of age.

*Effect of Supplementing the Rachitogenic Ration with a Small Amount of Vitamin D and Magnesium Compounds*

*Calf C-89.* This calf received the same ration as C-44 (1) which consisted of a grain mixture and wheat straw. C-89 developed a case of low calcium, normal phosphorus rickets by 462 days of age. The animal was also unable to use its hind quarters after this date. As indicated in Table 2, 5 per cent of magnesium carbonate was added to the ration at 464 days of age. The concentrations of calcium and inorganic phosphorus were 5.9 and 6.25 mg. per 100 cc. of plasma on this day. The change in the ration led to an immediate and considerable increase in the plasma calcium but only caused insignificant variations in the inorganic phosphorus. When this animal was slaughtered at 514 days of age, post-mortem examination revealed a fractured vertebra which had completely calcified and bones which failed to show the gross evidence of rickets.

The addition of magnesium supplements to the rachitogenic rations of C-99, C-101, C-104, C-107 and C-159 failed to prevent or to cure rickets in the absence of vitamin D but the marked response which had been observed in C-89 indicated the possibility that the ingested magnesium might have exerted a vitamin D sparing action. We (12) had previously demonstrated that 10 cc. of standard cod liver oil per day protected heifers from rickets to about 15 months of age after which time the plasma calcium and inorganic phosphorus values became subnormal. Five cc. of standard cod liver oil per day, which furnished insufficient vitamin D to meet the requirements, were added to the basal rachitic ration of 6 calves. The calcium, phosphorus and magnesium intakes of calves C-170 and C-173, which were fed the basal ration plus 5 cc. of cod liver oil, were approximately 10.0, 7.0 and 2.0 gm. per day respectively up to 6 months of age and approximately 16.5, 11.0 and 6.5 gm. per day from 6 to 10 months of age. C-174 and C-176

were fed the same ration supplemented with magnesium oxide and C-172 and C-175 received the same ration supplemented with magnesium carbonate. The calcium and phosphorus intakes were the same as for C-170 and C-173 but the magnesium intake was 7 gm. per day to 6 months of age and 16 gm. per day from 6 to 10 months of age.

*Calf C-170.* The body weights, plasma calcium, inorganic phosphorus and magnesium values appear in Table 3. During the first 4 months of age

TABLE 3

*C-170. Effect of the Rachitogenic Ration, Supplemented With 5 cc. of Cod Liver Oil Per Day, Upon the Plasma Calcium, Inorganic Phosphorus and Magnesium*

DATE	AGE	WT.	PLASMA			DATE	AGE	WT.	PLASMA		
			Ca	P	Mg				Ca	P	Mg
	<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>				<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>		
5-11-32	10	97	11.6	6.69	2.12	10-28	180	243	8.7	5.58	2.13
5-21	20	96				11-7	190	269			
5-31	30	103		5.81	1.99	11-17	200	286	8.7	6.54	2.57
6-10	40	111	12.6	7.02	2.14	11-27	210	301	10.5	6.69	2.93
6-20	50	118	13.6	5.74	2.62	12-7	220	326	9.5	7.53	2.75
6-30	60	128				12-17	230	345			
7-10	70	140	13.5	7.76	2.74	12-27	240	355	8.8	6.13	2.60
7-20	80	146	14.1	6.65	2.88	1-6-33	250	377	9.3	5.32	2.69
7-30	90	153				1-16	260 <sup>1</sup>	396			
8-9	100	161	13.2	6.38	2.45	1-26	270	411	8.1	6.41	2.62
8-19	110	178	11.9	7.35	2.51	2-5	280	429	7.3	5.71	2.58
8-29	120	189				2-15	290	448	8.3	5.12	2.33
9-8	130	194	12.2	5.95	2.69	2-25	300	462			
9-18	140	198	10.6	4.90	1.90	3-7	310	463	8.0	5.34	2.78
9-28	150	208		5.17	2.63	3-17	320	461	9.4	4.73	2.59
10-8	160	220				3-23	326	473			
10-18	170	230	9.6	5.10	2.08						

<sup>1</sup> Stiff at 266 days of age.

this calf had a few inorganic phosphorus values which were below 6.5 mg. per 100 cc. of plasma, whereas all of the calcium values maintained a normal position throughout this period. During the fifth and sixth months of age the inorganic phosphorus values were consistently below normal. They returned to normal during the seventh month, then progressively declined to a minimum of 4.73 mg. at the end of the survival period. The calcium values did not drop to below normal until the sixth month. They increased slightly during the seventh month, after which time subnormal values prevailed to the end of the experiment. The increased concentrations of calcium and inorganic phosphorus during the seventh month were associated with an increased appetite for grain, thereby causing an appreciable increase in the calcium, phosphorus and magnesium intakes. The plasma magnesium values also increased during this period and maintained a normal position until the end of the experiment. Rickets, as indicated by stiffness, were first observed at 266 days of age. The condition rapidly became more pronounced and by the time of slaughter at 326 days of age, the calf was very stiff.

*Calf C-173.* This calf grew at a slower rate and evidenced stiffness at an earlier age than C-170. The plasma calcium values were normal throughout the experiment, with few exceptions. The inorganic phosphorus values, however, were all below normal after 90 days of age. Stiffness was first observed at 220 days of age when the calcium and inorganic phosphorus values were 11.3 and 6.07 mg. per 100 cc. of plasma. The inorganic phosphorus continued to drop until at slaughter, at 336 days of age, it had reached a minimum of 3.90 mg. During the time when rickets were most pronounced, the plasma magnesium values were normal. The rachitic condition of this calf is shown in Plate I.

*Calf C-172.* This calf received the basal ration supplemented with magnesium carbonate until 11 months of age, at which time the carbonate was replaced by magnesium oxide. This animal was continued on experiment to determine the duration of the vitamin D sparing action of the magnesium oxide. The results are shown in Table 4. The plasma calcium and inor-

TABLE 4

*C-172. Vitamin D Sparing Effect of Magnesium Carbonate When Fed as a Supplement to the Rachitogenic Ration Plus 5 cc. of Cod Liver Oil Per Day*

DATE	AGE	WT.	PLASMA			DATE	AGE	WT.	PLASMA		
			Ca	P	Mg				Ca	P	Mg
	<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc</i>				<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>		
5-27-32	20	98	14.7	6.51	2.03	1 2-33	240	373			
6-6	30	108				1-12	250	410	11.5	7.81	2.91
6-16	40	115	14.6	8.23	2.29	1-22	260	408	11.2	8.39	2.75
6-26	50	121	13.8	7.81	2.39	2-1	270	425	10.3	7.40	2.53
7-6	60	131	13.5	4.50	1.95	2-11	280	446			
7-16	70	145				2-21	290	469	10.8	7.86	2.32
7-26	80	161	14.1	8.93	2.15	3-3	300	479	10.5	6.95	3.18
8-5	90	178	13.8	7.71	2.17	4-2	330 <sup>1</sup>	508	10.7	7.10	3.15
8-15	100	184				5-2	360	553	11.2	6.83	2.87
8-25	110	204	12.6	7.76	2.06	6-1	390	589	10.4	7.04	2.51
9-4	120	223	13.1	7.49	2.71	7-1	420	599	10.8	6.12	4.05
9-14	130	223	13.1	6.69	2.07	7-31	450	647	9.6	7.03	3.25
9-24	140	240				8-30	480	667	10.1	7.09	2.56
10-4	150	246		8.81	2.34	9-29	510	715	11.1	6.04	2.91
10-14	160	258	11.2	7.27	1.92	10-29	540	735	11.0	6.90	3.17
10-24	170	268				11-28	570	790	12.1	6.41	2.88
11-3	180	299	11.2	6.87	1.91	12-28	600 <sup>2</sup>	824	11.0	6.74	3.41
11-13	190	302	10.2	7.53	2.62	1-27-34	630	881	12.0	5.65	3.28
11-23	200	318	11.8	7.14	2.90	2-26	660 <sup>3</sup>	878	10.6	5.17	2.70
12-3	210	337				3-28	690 <sup>4</sup>	837	10.1	3.34	3.01
12-13	220	353	11.9	8.33	2.08	4-16	709 <sup>5</sup>		9.5	2.35	2.67
12-23	230	369	12.2	7.44	2.82	4-23	716 <sup>6</sup>		8.9	2.60	2.73

<sup>1</sup> Changed to MgO. <sup>2</sup> First evidence of stiffness. <sup>3</sup> Stiff. <sup>4</sup> Hocks enlarged. <sup>5</sup> Calved.

<sup>6</sup> Died.

ganic phosphorus values were normal, with very few exceptions, to 20 months of age. The normal condition of this animal is shown in Plate I. The clinical evidence of rickets coincided with the first manifestation of stiffness at 602 days of age. The calcium and inorganic phosphorus values

were 11.0 and 6.74 mg. per 100 cc. of plasma at 600 days of age. This animal developed all of the clinical symptoms of rickets yet the plasma calcium and inorganic phosphorus values remained within the normal limits. Following the first evidence of stiffness, however, the inorganic phosphorus steadily dropped. C-172 was bred at 447 days of age. The demand for additional vitamin D for gestation undoubtedly aggravated the rachitic condition. Both the calcium and inorganic phosphorus had decreased to 9.5 and 2.35 mg. at the time of calving. C-172 died 7 days following calving, at which time these values were 8.9 and 2.60 mg.

*Calf C-175.* This calf also received magnesium carbonate as a supplement to the rachitogenic ration. The inorganic phosphorus values were within the normal range throughout the entire experiment. The plasma calcium values were also normal with the exception of the four terminal values which were only slightly subnormal. The terminal calcium, inorganic phosphorus and magnesium values were 9.4, 8.50 and 3.31 mg. per 100 cc. of plasma respectively. This animal did not manifest stiffness at any time. It was slaughtered at 320 days of age.

*Calf C-174.* This calf received the basal ration supplemented with magnesium oxide and was kept on this ration to determine the duration of the effectiveness of the magnesium supplement in preventing rickets. The condition of this animal at 404 days of age is shown in Plate I. C-174 was bred at 448 days of age and conceived at this service but she aborted at 524 days of age. The anorexia, which was associated with the premature expulsion of the fetus, exaggerated the rachitic condition. Stiffness was first observed at 583 days of age. The plasma calcium values were all within the normal range during the experiment and only 6 subnormal inorganic phosphorus values were observed from birth to 500 days of age. After this age the inorganic phosphorus slowly declined to a minimum of 3.93 mg. per 100 cc. of plasma. C-174 developed normal calcium, low inorganic phosphorus rickets. She was slaughtered at 602 days of age, at which time the plasma calcium, inorganic phosphorus and magnesium values were 11.9, 5.43 and 3.08 mg. respectively.

*Calf C-176.* This calf received the basal ration supplemented with magnesium oxide. The results are shown in Table 5. Almost all of the plasma calcium and inorganic phosphorus values were within the normal range. There were only two calcium values below 10.0 mg. per 100 cc. (9.6 and 9.9 mg.) and only two inorganic phosphorus values were below 6.0 mg. (5.17 and 5.48 mg.).

There was no evidence of stiffness. To all outward appearances this animal was normal at the time of slaughter at 317 days of age. The terminal plasma calcium, inorganic phosphorus and magnesium values were 10.8, 6.41 and 3.18 mg. respectively.

Post-mortem examination of the long bones of the animals receiving a

TABLE 5

*C-176. Vitamin D Sparing Effect of Magnesium Oxide When Fed as a Supplement to the Rachitogenic Ration Plus 5 cc. of Cod Liver Oil Per Day*

DATE	AGE	WT.	PLASMA			DATE	AGE	WT.	PLASMA		
			Ca	P	Mg				Ca	P	Mg
	<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>				<i>days</i>	<i>lbs.</i>	<i>mg. per 100 cc.</i>		
6-18-32	20	108	13.8	8.87	2.40	11-25	180	277	10.6	7.58	2.80
6-28	30	110	15.0	8.83	2.47	12-5	190	296			
7-8	40	121				12-15	200	311	11.2	7.71	2.74
7-18	50	131	13.9	7.62	2.36	12-25	210	328	11.8	6.22	2.62
7-28	60	142	13.3	6.79	2.07	1-4-33	220	345	11.5	7.35	3.31
8-7	70	158				1-14	230	367			
8-17	80	176	13.9	7.14	1.98	1-24	240	373	10.8	6.28	2.60
8-27	90	177				2-3	250	399	9.9	6.38	3.06
9-6	100	202	12.9	6.72	2.37	2-13	260	416			
9-16	110	215	13.8	8.62	1.63	2-23	270	433	10.9	6.87	2.44
9-26	120	233				3-5	280	440	10.7	6.69	3.68
10-6	130	237		8.33	2.91	3-15	290	434	10.5	6.22	2.67
10-16	140	230	9.6	5.17	1.56	3-25	300	453			
10-26	150	228	10.3	5.48	2.17	4-4	310	459	11.2	6.79	3.52
11-5	160	250				4-11	317	470	10.8	6.41	3.18
11-15	170	262	11.4	6.69	2.74						

TABLE 6

*Ash and Percentage Composition of Bones and Terminal Plasma Values of Calves which Received the Rachitogenic Ration, Supplemented with Magnesium Carbonate or the Oxide, With and Without Cod Liver Oil*

CALF	BONE	AGE	WT.	ASH	Ca	P	Mg	PLASMA		
								Ca	P	Mg
<i>No.</i>		<i>days</i>	<i>lbs.</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>mg. per 100 cc.</i>		
C-170	8th	323	473	45.36	17.41	8.10	0.49	9.4	4.73	2.59
C-173	right	319	323	44.98	17.75	8.18	0.66	9.0	3.90	2.93
C-172	rib	718	837	49.60	19.98	9.06	0.80	8.9	2.60	2.73
C-172's		0 <sup>1</sup>	74	47.60	18.20	8.71	0.51			
C-174		602	740	50.80	19.60	9.05	0.82	11.9	5.43	3.08
C-175		318	565	50.90	19.37	9.24	0.74	9.4	8.50	3.31
C-176		317	470	49.45	19.80	9.12	0.66	10.8	6.41	3.18
C-99	Orbital	256	255	47.61				6.9	6.83	2.53
C-104	Plate	194	244	45.77				7.7	5.14	2.13
C-170				46.80	18.71	8.35	0.57			
C-173				44.20	17.43	7.94	0.66			
C-89		514	down	50.82				9.5	6.28	
C-174				53.85	21.00	9.56	0.80			
C-175				53.35	20.91	9.52	0.91			
C-176				51.00	20.45	9.08	0.86			
C-99	Dental			48.96						
C-104	Pad			47.89						
C-107		206	154	42.51				9.7	2.35	2.47
C-170				46.86	17.35	8.27	0.58			
C-173				46.95	18.59	8.16	0.63			
C-89				54.79						
C-174				54.50	21.25	9.62	0.95			
C-175				50.25	19.50	8.77	0.72			
C-176				50.20	20.30	8.86	0.75			

<sup>1</sup> Full term calf, stillbirth.

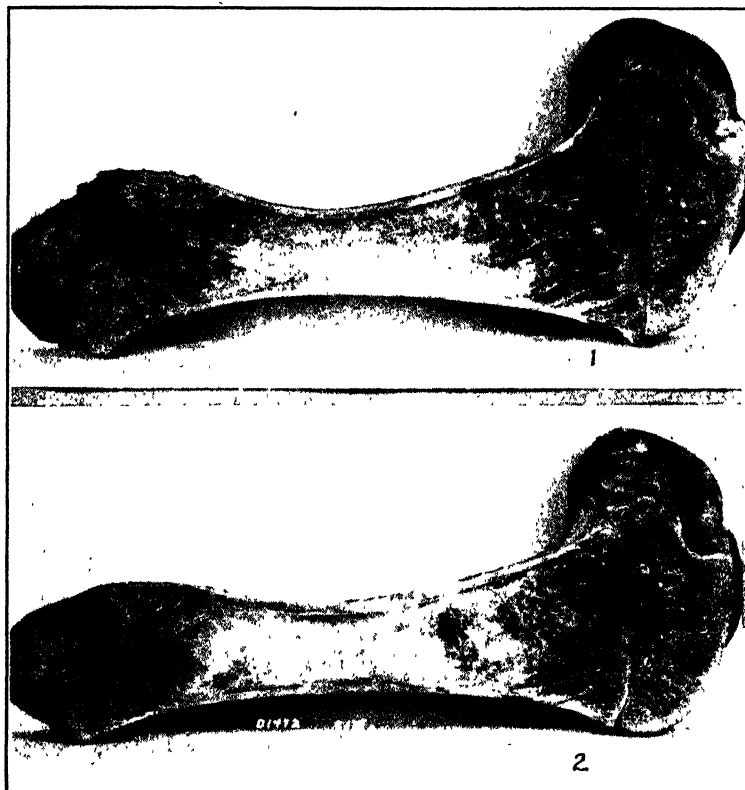
magnesium supplement showed these bones to be in almost as poor condition as similar bones from the animals which did not receive a magnesium supplement. There was a pronounced difference, however, between the two groups when the costochondral junctions were compared. In C-170 and C-173 these junctions had considerable uncalcified osteoid tissue while in C-175 and C-176 these areas were well calcified, yet upon gross examination, the ribs of the two latter animals appeared to be soft. The ash values for the 8th rib, orbital plate and dental pad were higher for the latter two animals than for the two calves which did not receive a magnesium supplement. The ash and mineral analyses are summarized in Table 6. Plate II shows the lack of trabeculation in the femur of C-170 and the improved trabeculation in the femur of C-175.

#### DISCUSSION

It was evident from the results with calves C-99, C-101, C-104, C-107 and C-159 that the addition of either magnesium carbonate or the oxide to the rachitogenic ration failed to produce antirachitic effects. These results are in agreement with other workers (7) (8) who reported that the addition of magnesium compounds to rachitogenic diets either failed to cure rickets or interfered with normal bone formation. The results with C-89, however, indicated that the addition of magnesium carbonate to a ration containing wheat straw aided in restoring the blood calcium to normal and also revealed at autopsy, the complete calcification of a previously fractured vertebra. Oat straw has been shown to contain some vitamin D (13). Both C-170 and C-173, which received the rachitogenic ration plus 5 cc. of cod liver oil, developed marked rickets, which were indicated by a prompt and continuous decline in the concentrations of calcium and inorganic phosphorus in the plasma and by stiffness. The addition of magnesium supplements to the rachitogenic rations of C-172 and C-175 ( $\text{MgO}$ ) and C-174 and C-176 ( $\text{MgCO}_3$ ) demonstrated that these supplements were only effective in the prevention of rickets when a small amount of vitamin D was present in the ration.

The magnesium supplements aided in maintaining the normal concentrations of calcium and inorganic phosphorus in the blood of the calves up to 10.5 months of age when 5 cc. of cod liver oil per day were added to the ration. There was also a tendency, although not marked, for the concentration of magnesium to increase in the plasma, but the concentration never increased sufficiently to produce drowsiness. Hirschfelder (14) reported that coma set in when the concentration of magnesium reached 17 mg. per 100 cc. and that a number of patients showed a decided drowsiness or even slight coma when the concentrations were only about two-thirds of this value. There is a slight possibility that the moderate increase in the plasma magnesium may have reduced the sensation of pain so that stiffness did not take

## PLATE II



1. Lack of trabeculae in a femur from C-170.
2. Improved trabeculation in a femur from C-175.

place in these calves. There were no manifestations of clinical rickets by these animals up to 10.5 months of age but the rachitic conditions of C-170 and C-173 were severe when these calves were slaughtered at the above age.

The two animals, C-172 and C-174, which were kept on the rachitic ration supplemented with 5 cc. of cod liver oil per day and a magnesium supplement, did not become stiff until about 20 months of age. In the case of these young cows, whose skeletons could not have been as completely calcified as fully mature cows, the mineral drain in favor of the fetus was especially serious. The degree of calcification of C-172's calf was not appreciably modified by the apparent lack of vitamin D as the ash and mineral analyses of the 8th rib were less than 10 per cent below the normal values for a new-born calf.

Although the plasma calcium and inorganic phosphorus values and the absence of stiffness of the calves fed the magnesium supplements indicated better calcium and phosphorus utilization, yet their bones appeared soft. The long bones appeared to be in almost as poor condition as those from the calves receiving the rachitogenic ration without the magnesium supplement. Plate II illustrates the condition and amount of trabeculae in a comparative longitudinal section of a femur from each group. The ash and mineral values of the 8th rib, orbital plate and dental pad would also indicate improved utilization of calcium and phosphorus due to the ingestion of the magnesium supplements. The differences in the ash values were not due to differences in growth because C-170 and C-173 gained 360 and 256 pounds during the first 300 days of age while C-172, C-174, C-175 and C-176 each gained 376, 398, 451 and 353 pounds respectively during the same period. The rapid growth of C-175 probably accounts for the subnormal plasma calcium values toward the end of the experiment.

Our results indicate that some magnesium compounds have a vitamin D sparing effect. This sparing action may be due to increased solubility of calcium in the intestines since Forbes (15) reported that magnesium increased the solubility of calcium *in vitro*. It may be inferred that magnesium may be substituted for calcium in the excreta and by this means promote calcium utilization. The mechanism of the magnesium action may also be due to its effect upon phosphatase activity as suggested by von Euler and Rydholm (2) and Kay (3). Hommerberg (16) reported that with low concentrations of phosphatase, a certain amount of magnesium was necessary to activate the enzyme. He also stated that the phosphoric acid esters seemed to decompose spontaneously under the influence of magnesium.

From the fact that these magnesium compounds exerted a definite vitamin D sparing effect, it may be indicated that the magnesium content of feeds should be taken into consideration when evaluating their antirachitic potency. Legume hays and corn silage may exert a greater antirachitic influence than their vitamin D content would indicate.

#### SUMMARY AND CONCLUSIONS

1. The addition of magnesium carbonate to a rachitogenic ration failed to prevent the manifestation of clinical rickets in calves.
2. The addition of magnesium carbonate to the ration of C-89, after the onset of rickets, caused an increase in the plasma calcium and promoted the calcification of a fractured vertebra. Four pounds of wheat straw furnished the only source of vitamin D.
3. The rachitogenic ration fed to calves C-170 and C-173 supplemented with 5 cc. of cod liver oil per day did not protect these calves from rickets.
4. The addition of one per cent—on the dry-matter basis—of magnesium carbonate to the rations of calves C-172 and C-175 prevented the clinical

symptoms of rickets to 10.5 months of age. C-172 had normal plasma calcium and inorganic phosphorus values, while C-175 had normal inorganic phosphorus values and a few subnormal terminal calcium values during this period.

5. When the same level of magnesium oxide was added to the rations of C-174 and C-176, no clinical evidence of rickets was observed. The plasma calcium and inorganic phosphorus values were normal up to 20 months of age for C-174 and to 10.5 months of age for C-176. C-172 which received magnesium oxide in place of magnesium carbonate after 11 months of age had normal calcium and inorganic phosphorus values to 20 months of age.

6. The ash and mineral values of the bones indicated better calcium and phosphorus utilization due to the ingestion of magnesium supplements.

7. The results of this experiment indicate that the magnesium content of feeds for dairy cattle may contribute to their antirachitic effect.

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# THE EFFECT OF SEASON OF THE YEAR AND ADVANCING LACTATION UPON MILK YIELD OF JERSEY COWS

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## INTRODUCTION

Advancing lactation long has been observed to decrease daily milk yield of dairy cows, but the seasonal influence upon daily milk yield has been studied little, mainly for lack of a satisfactory method of analysis. Such a method was applied recently at the Florida station, in a study of variations in the butterfat tests of Jersey milk (3). The same method has been applied to an analysis of daily milk yields.

## THE STATION HERD AND ITS ENVIRONMENT

The Florida station dairy herd earlier consisted of both registered and high grade Jersey cows, the majority of which were bred and raised on the farm. As described recently (3), the herd is located in the middle of the north end of the Florida peninsula, under an environment which varies less than does that of many dairy regions, due to proximity of large bodies of water and the general direction of the prevailing winds. Feeding practises have been relatively uniform over the period of years for which records were studied (1917 to 1932, inclusive), except for modifications in mineral content brought about mainly by inclusion of bone-meal in the concentrates. The rations and feeding practises followed were described in Florida station technical bulletin 262.

## REVIEW OF LITERATURE

The influence of the season of freshening on total milk yield has been the object of a number of investigations. McDowell (13) assembled records of 64 cow testing associations between 1910 and 1920, comprising 10,870 cow-years. He found that the fall-fresh cows were the highest producers, followed by those calving during winter, summer and spring. Cannon (4) tabulated 68,000 records of Iowa dairy herd improvement association cows of all breeds for the years 1925 to 1930, and noted that the cows fresh during November produced the most milk, and those fresh during June yielded the least milk during the year. Wylie (18) analyzed 2,900 Jersey Register of Merit records completed in the United States in 1921, and noted that those Jerseys fresh in July produced the most, and those calving in November were the next highest in milk production. The group of cows that calved during August were lowest in milk yields.

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Hogstrom (12), using 891 lactations mainly by Ayrshire and grade Ayrshire cows in Sweden, observed the highest milk yields among cows calving in August, and the lowest in the group fresh in November. Headley (11), in Nevada, studied 124 lactations by Holstein cows fed uniformly throughout the year, and noted that the highest and lowest yielding groups freshened in winter and in September, respectively. Hammond and Sanders (10) found in England that the highest milk yields were obtained from cows calving in the autumn, and the minimum yields from those fresh in July, based on lactation records of four recording societies, with animals of eight breeds. Sanders (14) also found the highest milk production with cows that calved during October, November and December.

The decline in rate of secretion due to advancing lactation has been observed rather widely. Eckles (7) points out that the second month in lactation is the highest in milk production. From this point to the eighth month the decline is gradual, but it is more rapid from the ninth to twelfth months for cows calving annually. Cows not bred to calve within the year, as sometimes is practised on official test, do not show as marked a decline after the eighth month, due to delayed breeding. Carlyle and Woll (5) found on the average, an eight per cent decrease in milk yield for each month during the progress of the lactation period, in a study involving five breeds.

Woll (17) studied the lactations of 323 cows of three breeds, and found that the monthly decline in average daily milk yield was 2.3 pounds between the first and eighth months. From the ninth to the twelfth months, the decline was at the rate of 3.3 pounds, or a 43 per cent greater rate of decline in the daily milk production near the close of the lactation. Beach (2) showed the average monthly decrease from the first to the eighth months to be 6.5 per cent, but double that rate from the eighth to the tenth month after calving. Turner (16) studied 3,215 Guernsey Advanced Registry lactation records, and found the average daily production to be 34.3 pounds of milk in the second month; 23.7 pounds in the eighth month and 17.9 pounds in the twelfth month. With 305 Jersey Register of Merit records in Missouri, maximum daily milk yield was 30.0 pounds in the first month after calving; 20.6 pounds in the eighth month and 17.3 pounds the twelfth month. Ninety-five Holstein records in the station herd attained a maximum average daily milk yield of 40.3 pounds in the second month; 29.3 pounds in the eighth month and 20.1 pounds in the twelfth month. With cows under official testing conditions, breeding often is delayed in order that the inhibitory effect of advancing gestation may affect the record as little as possible. This may account for the lower rate of decline after the eighth month.

The seasonal influence on daily milk production has not been studied apart from other modifying factors. Many investigators have made obser-

vations similar to Farrington's (8), that milk yield increased when cows were changed from the stable onto spring pastures.

#### EXPERIMENTAL METHODS

Milk records of registered and high grade Jersey cows in the station dairy herd between 1917 and 1933 were assembled by ten-day periods, using the date of calving as the first day of the lactation. These were computed to uniform age basis, using the factors of Clark (6) for Jerseys milked twice daily. Lactations were grouped according to the month of freshening, and by seasons of the year, as defined previously for this latitude (1).

Statistical significance and the relationship of the variance arising from the advance in lactation and from milk yield at different seasons of the year, were determined by application of Fisher's "Z" test (9), as modified by Snedecor (15). The 144-cell arrangement of milk production by months, by groups of cows calving during each month of the year, allowed an even distribution of one factor throughout the data while making an analysis for the second factor. (Illustrated in Table 2).

#### PRESENTATION OF RESULTS

##### *Season of freshening*

The 319 lactations available for this study showed an average production per lactation of 5,262 pounds of milk, computed to maturity. The distribution of these lactation records according to season of calving, was reasonably uniform, as shown in Table 1. The differences in average milk yield between

TABLE 1  
*Average Milk Yield by Jersey Cows Calving at Different Seasons of the Year in Florida*

SEASON	NUMBER OF LACTATIONS	AVERAGE MILK YIELD	PERCENTAGE OF AVERAGE LACTATION
Spring	74	4,863.6	92.42
Summer*	104	5,339.0	101.45
Autumn*	70	5,435.7	103.29
Winter	71	5,445.1	103.47
Average	319	5,262.6	100.00

\* Because of the latitude and environment, "seasons" in Florida have been defined on the basis of the average dates of first and last killing frosts of winter (3 months), and on the duration of the rainy season in summer. On this basis, Summer includes June, July, August and September, and Autumn consists only of October and November.

groups, however, were not significant. It may be recalled in this connection that the seasonal variations in temperatures fluctuate within a narrow range in the Florida peninsula, because of its proximity to the Gulf of Mexico and the Atlantic Ocean.

TABLE 2  
*Effect of Advancing Lactation and of Season of the Year Upon Milk Yield in 319 Lactations by Jersey Cows at the Florida Agricultural Experiment Station, 1917 to 1932*

MONTH AFTER CALVING	Average milk yield in pounds by 30-day periods for cows calving in the month of:												AVERAGE
	JAN.	FEB.	MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	
First	707.7	583.0	665.9	653.8	644.1	699.6	704.4	647.9	639.1	686.8	663.2	678.4	664.6
Second	683.6	739.5	607.2	680.6	689.3	662.2	741.6	708.1	640.8	606.2	628.6	677.0	672.0
Third	649.0	622.7	690.5	553.0	642.3	653.5	583.7	667.2	652.6	544.1	522.4	560.3	611.8
Fourth	524.1	602.8	589.8	649.4	524.1	599.4	572.5	515.7	633.1	589.3	457.3	453.8	559.3
Fifth	437.1	485.8	548.8	564.9	633.4	507.5	555.9	504.5	462.0	573.8	481.2	394.8	512.5
Sixth	375.0	402.7	453.1	525.2	542.9	598.9	443.6	495.2	456.0	363.5	485.1	419.9	463.4
Seventh	386.8	382.7	374.2	437.4	515.5	513.3	534.3	394.0	424.7	385.4	275.9	456.9	421.8
Eighth	438.5	387.4	319.4	358.8	404.6	483.2	438.4	451.0	343.1	335.6	318.9	216.1	374.6
Ninth	178.5	392.7	382.7	260.9	321.7	373.3	387.6	332.1	387.2	276.6	244.8	254.7	311.9
Tenth	210.5	139.3	324.5	286.1	223.7	278.8	276.5	273.1	245.1	272.7	169.9	217.0	243.1
Eleventh	180.7	132.3	97.6	279.6	269.0	182.1	192.5	151.8	181.2	179.3	196.6	99.3	178.5
Twelfth	60.8	124.9	83.6	89.7	255.3	222.4	136.9	109.9	97.6	113.2	118.9	140.0	129.4
Average	402.7	414.7	423.9	445.1	472.2	481.2	464.0	437.5	430.2	410.5	380.2	380.7	5,142.9

## ADVANCING LACTATION AND SEASONAL INFLUENCE ON MILK YIELD

The decline in milk production with advancing lactation and the rate of milk production in the different seasons of the year were measured by assembling the data for an analysis of variance (15). Individual milk yields were assembled by consecutive 30-day periods for the cows that freshened in the several calendar months. The calculated average milk yields thus do not represent *exact calendar months*, which vary from 28 to 31 days in length. Instead, they are 30-day periods for the groups of cows that freshened in these months, and in sufficient numbers that the records involved come very close to centering near the middle of each calendar month. Thus, the monthly milk yields were analyzed for both rate of production in consecutive months after calving, and also for calendar months throughout the year, irrespective of dates of calving. The results of this analysis are shown in Table 2.

The rate of decline with advancing lactation was measured with an even distribution of the seasonal influence throughout the data. Based on the previous month's milk production, the rates of decline in milk secretion after the second month in lactation were calculated to be 8.96, 8.58, 8.37, 9.58, 8.98, 11.33, 16.74, 22.06, 26.57 and 27.51 per cent, respectively. This shows a relatively uniform rate of decline from the second through the

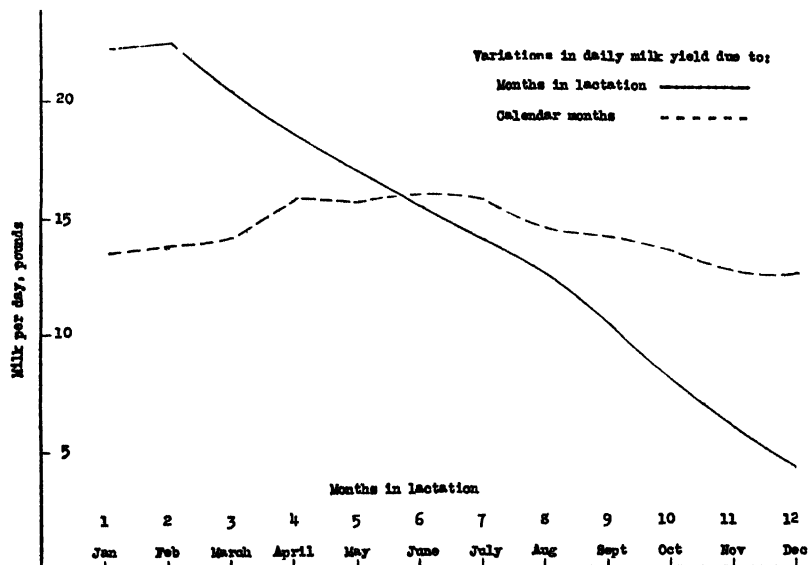


FIG. 1. The average lactation curve above is based on a study of 319 normal lactations of Jersey cows milked twice daily in the Florida station herd. Seasonal variation in milk production in the local environment at this latitude (30° N.) was only one-fourth the magnitude of the variation due to advancing lactation.

seventh month, and a progressively greater rate thereafter. This greater rate of decline in milk secretion in the later months of lactation has been shown by many investigators to be associated with the depressing influence of advancing stage of gestation.

Advancing stage of lactation was a far greater factor affecting milk yield than was season of the year, as seen in Figure 1, and in the analysis of variance in Table 3.

TABLE 3  
*Sources of Variance and Significance of Variations Affecting Monthly Milk Yields of Jersey Cows*

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	"F" VALUE
Total	144 - 1 = 143	5,015,722.35		
Between months after calving	12 - 1 = 11	4,522,949.42	411,177.22	147.46
Between calendar months	12 - 1 = 11	145,389.65	13,217.24	4.74
Remainder	11 × 11 = 121	337,383.27	2,788.29	

The seasonal influence on milk production, irrespective of advancing lactation, acts within a relatively narrow range in the environment of Florida. The low average monthly milk production occurred during November and December, and the high average monthly milk production in June. The change in rate of production was gradual between these points.

TABLE 4  
*The Relationship Between Mean Temperatures, Average Rainfall and Monthly Milk Production of Jersey Cows at the Florida Station, 1917 to 1932*

MONTH	MEAN TEMPERATURE			AVERAGE PRECIPITATION	AVERAGE MILK PRODUCTION
	Maximum	Minimum	Average		
	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>inches</i>	<i>pounds</i>
January	68.7	46.5	57.6	3.15	402.7
February	71.7	48.7	60.2	2.83	414.7
March	75.4	52.8	64.1	3.62	423.9
April	81.4	57.4	69.4	3.23	445.1
May	86.5	62.5	74.5	3.46	472.2
June	89.7	68.8	79.25	6.96	481.2
July	90.6	70.8	80.7	9.07	464.0
August	90.7	70.8	80.75	6.27	437.5
September	89.0	69.1	79.05	5.36	430.2
October	82.9	61.2	72.05	2.56	410.5
November	74.6	51.4	63.0	1.94	380.2
December	69.5	47.6	58.55	2.59	380.7
Total				51.04	5,142.9

Milk yields began to decline before the highest mean temperature was attained early in the summer rainy season.

Moisture and warm temperatures favor the growth of pasture grasses. The relationship between grass growth and milk secretion was not considered. Agronomic data are necessary before this relationship can be studied. The average mean temperature, rainfall and milk yields by month, of Jersey cows in the Florida station herd for the years 1917 to 1932, inclusive, are shown in Table 4.

#### SUMMARY AND CONCLUSIONS

The relatively uniform environment of Florida was not conducive to significant differences in yearly milk production between groups of Jersey cows calving during the different seasons of the year.

Advancing stage of lactation, irrespective of season of the year, produced a relatively uniform rate of decline in monthly milk yield from the second through the seventh month in milk. The progressively greater rate of decline after the seventh month is associated with the inhibiting factor of advancing gestation.

The seasonal influence on milk yield, irrespective of advancing lactation, resulted in maximum rate of milk production during June, and the minimum during November and December. The peak of milk production was attained in advance of the mean maximum temperature early in the summer rainy season.

#### ACKNOWLEDGMENT

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# JOURNAL OF DAIRY SCIENCE

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## OBSERVATIONS ON THE FREEZING OF MILK AND CREAM

### I. THE EFFECT OF FAT CONCENTRATION UPON THE DIS- TRIBUTION OF CONSTITUENTS IN THE FROZEN AND UNFROZEN PORTIONS OF PARTIALLY FROZEN MILK AND CREAM\*

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#### FOREWORD

In the freezing of milk and cream pure ice separates from the super-cooled liquids in the form of spiny crystals, which, without agitation, usually build up into definite structures. To separate the ice quantitatively from the liquids is almost a physical impossibility since the crystal structures enmesh, by capillarity and obstruction, considerable quantities of the liquids and their components. Consequently, in any separation of the unfrozen and frozen portions of partially frozen milk and cream, short of washing, the frozen portion always contains, in addition to the congealed substance (water), considerable portions of the liquid phase held physically. This is generally recognized and the terms "frozen portion" and "unfrozen portion" as used in this paper are subject to this interpretation. Furthermore, it is realized that milk and cream theoretically would not freeze totally until a temperature lower than the cryohydric point of the ingredient having the lowest cryohydric point is reached. They become apparently totally frozen, however, when no liquid drains from the icy structure and the products are solid masses exhibiting no leakage even when broken up. It is in this sense that the term "total freezing" is commonly used and it is so used in this paper.

It is generally known that as milk freezes the constituents are progressively concentrated in the unfrozen or liquid portion leaving the frozen or

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congealed portion depleted. This was shown as early as 1886 by Veith (1) and has been substantiated numerous times since. A possible exception to this statement might be made relative to the milk fat. The literature exhibits contradictory findings in this respect (2, 3, 4, 5, 6, 7, 8). Some investigators show data indicating that the fat is more concentrated in the unfrozen liquid; others show it to be higher in the frozen portion; and still others obtained contradictory results in different trials. The consensus of opinion seems to be that the degree of freezing, coupled with the time required for freezing (during which interval creaming takes place) influences markedly the distribution of fat between the frozen and unfrozen portions. Henzold (9) found that freezing with mild agitation always produced a higher fat and solids content in the unfrozen portion compared with freezing without agitation. The variations in the distribution of fat and other ingredients between the frozen and unfrozen portions of partially frozen milk and cream have never been systematically studied over the entire range of freezing.

Until quite recently it has been assumed that cream freezes similarly to milk. However, Baldwin and Combs (10) and later Trelogan and Combs (11) found that cream containing 25–30 per cent of fat or over freezes homogeneously so far as the fat is concerned and that an analysis of a sample chipped from the surface of a solidly frozen block will give results checking in per cent of fat with the original unfrozen cream.

The last mentioned workers (12) attribute this homogeneity to the fact that cream of this richness exhibits practically no evidence of fat-rising when aged at low temperatures. This question then arises: Does such cream freeze homogeneously with respect to the other ingredients as well as the fat, and if so, where is the transition point between homogeneous and heterogeneous freezing?

The purpose of the investigation reported here was to study systematically the progressive freezing of milk and cream, over the entire range to total freezing, hoping thereby to explain some of the apparent inconsistencies in the literature relative to fat distribution; to determine whether cream of 25 per cent fat content and over freezes homogeneously with respect to the ingredients other than fat; and, to note the possible effect of the fat phase on the phenomenon.

## EXPERIMENTAL

### *Methods*

Skimmilk and whole milk and cream mixtures were standardized to contain 0 per cent, 4 per cent, 13–15 per cent and 25 per cent of fat respectively and were frozen undisturbed in 2-quart covered tin containers in still air at  $-23$  to  $-28^{\circ}$  C. until they had congealed to varying degrees. Upon removal from the freezing chamber the unfrozen portion was immediately

carefully strained through a coarse cheese cloth. Any ice crystals remaining on the cloth were put with the frozen portion and the latter was thawed in a water bath maintained at from 40° to 45° C. until no ice remained. Analyses for the amount of fat, total solids, and titratable acidity, and determinations of the freezing point and the pH were then made on the frozen and unfrozen portions.

Milk fat was determined by the Babcock method, total solids by the Mojonnier method, titratable acidity according to the method of the A. O. A. C. (13), the freezing point using a Hiortvet cryoscope (13), and the pH with a Leeds and Northrup potentiometer using the quinhydrone electrode.

The degree of freezing was determined by weighing the frozen portion, and is stated in terms of the percentage by weight of the original.

The results are averages of from three to twelve trials at each percentage frozen for which a point is recorded on the graphs.

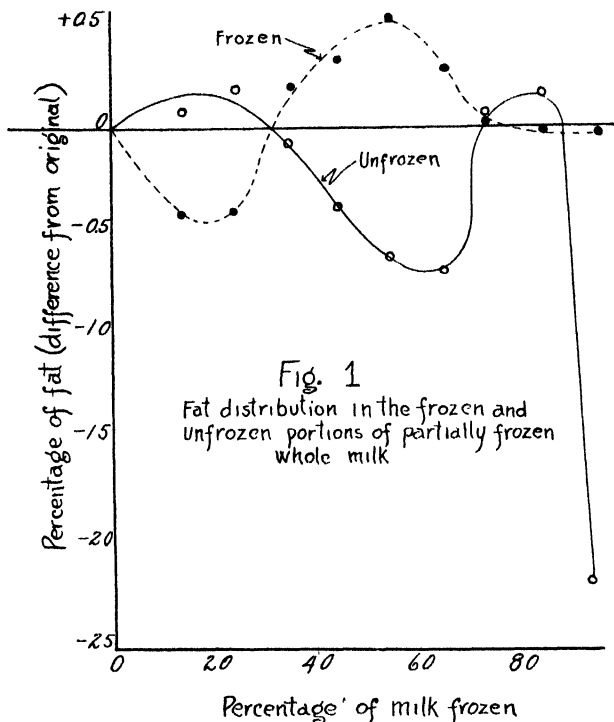
In order to make all of the results comparable, the analyses of the original samples (prior to freezing) were used as a reference and the results obtained for the frozen and unfrozen portions are recorded as the differences (either plus or minus) from the original.

Under the conditions used, the time to freeze to 100 per cent (apparently solid) was approximately 12 hours. The time was reduced somewhat as the fat content of the samples increased. The percentage frozen was a linear function of the time up to about 60 per cent frozen after which freezing was less rapid.

#### *Distribution of Fat in Partially Frozen Milk*

When fluid milk containing 4 per cent of fat was partially frozen, analyses of the frozen and unfrozen portions made at intervals from the time freezing became evident until almost completely frozen, showed that the fat percentage of the two portions varied considerably and inversely over the entire range of freezing. Figure 1 shows the average results obtained in this study. At the start of freezing the fat percentage of the frozen portions was lower than the original, but after approximately 35 per cent of the fluid was frozen, the fat percentage was higher than the original, and remained higher until approximately 75 per cent was frozen when the difference between the frozen portion and the original became negligible, and remained so until the milk was entirely frozen. The fat content of the unfrozen portion behaved in inverse fashion up to the time about 85 per cent of the milk was frozen after which with further freezing the fat content decreased sharply.

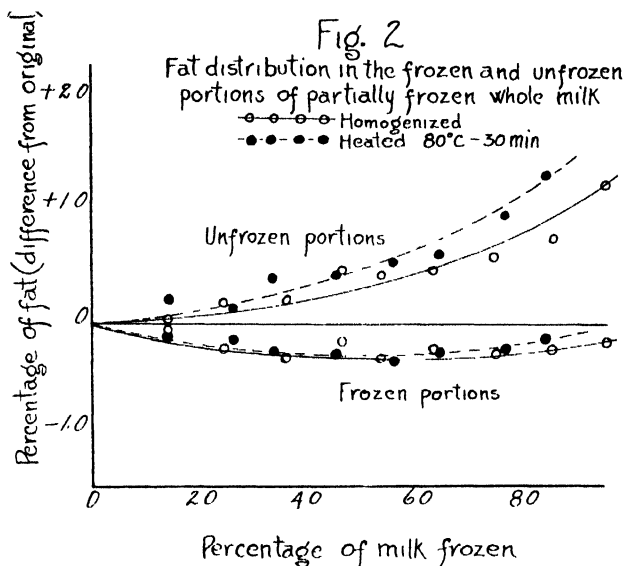
A study of the cross section of a frozen block of milk indicated that creaming of the milk occurred during freezing and suggested that the variable analytical results obtained were probably related to the speed of



freezing (particularly of the surface), the rapidity and completeness of creaming and the size and shape of the container. Considering the results obtained, it is not to be wondered at that the data in the literature show such wide diversity.

In order to prove that the analytical results shown in Figure 1 are caused by the creaming phenomenon of the milk, two other series of samples were frozen in similar fashion. These samples were treated to destroy the creaming tendency by homogenization at 2500 pounds pressure and by heating the milk to 80° C. for 30 minutes prior to freezing. The average results obtained in this study are plotted in Figure 2. When the creaming of the milk was prevented by either method, the fat percentage of the unfrozen portion increased progressively over the course of freezing, while that of the frozen portion decreased at first but as the solidly frozen condition approached, the fat percentage increased to nearly that of the original unfrozen milk as would be expected.

These results show that the creaming tendency of milk, frozen without agitation, is the factor responsible for the variable fat distribution in the frozen and unfrozen portions of the product.



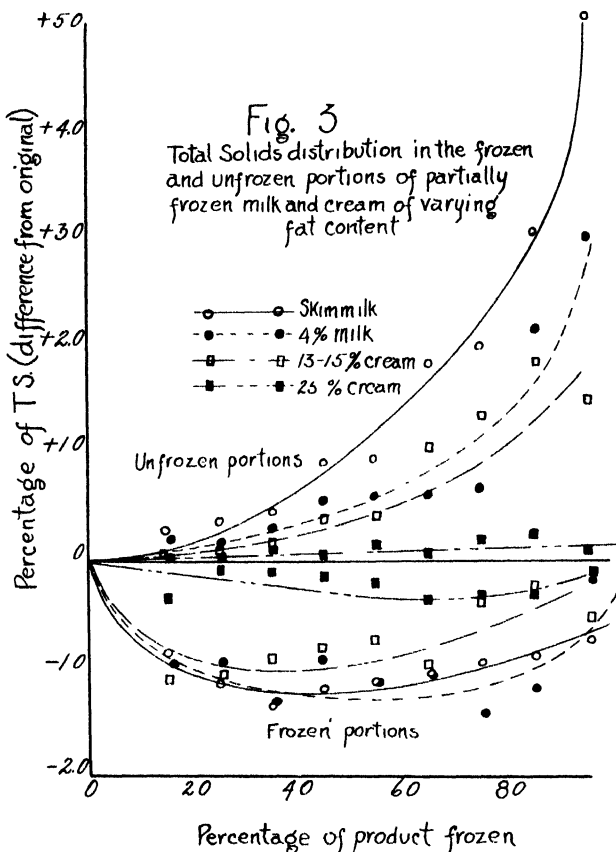
#### *Distribution of Total Solids in Partially Frozen Milk and Cream*

It has been shown by other investigators (10), (11), (12), and confirmed again by us that cream containing more than about 25 per cent of fat freezes practically homogeneously so far as the fat is concerned. Whether this also holds true for the other ingredients of such cream, however, has not been previously determined.

The effect of increasing fat concentration upon the distribution of the total solids constituents in the unfrozen and frozen portions of partially frozen milk and cream was studied using skim milk, 4 per cent milk, 13-15 per cent cream and 25 per cent cream. The average results obtained in several trials are presented in Figure 3. These demonstrate that as the fat concentration of the milk or cream increases, the tendency toward diffusion or concentration of the total solids of milk into the unfrozen portion decreases. The cream containing 25 per cent of fat exhibited almost homogeneous distribution of the total solids throughout the entire course of freezing. It would seem, therefore, that all of the constituents of cream containing more than 25 per cent of fat are distributed homogeneously between the frozen and unfrozen portions of such cream when freezing is allowed to progress without agitation.

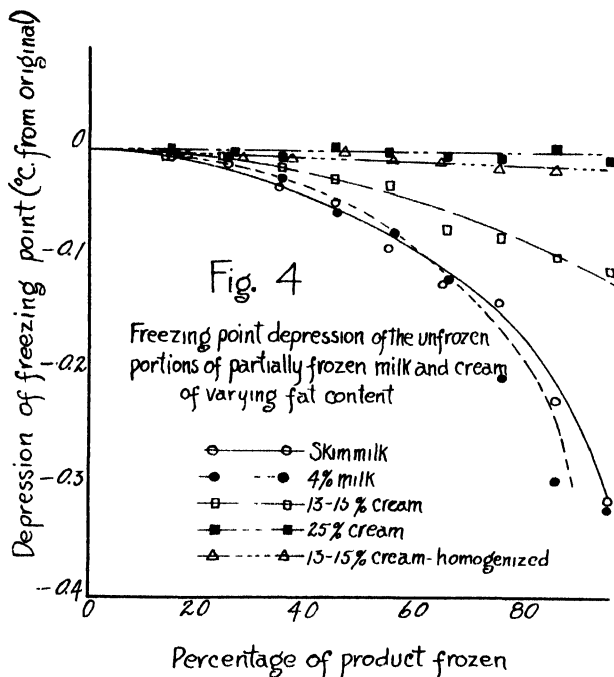
#### *Distribution of the Soluble Constituents in Partially Frozen Milk and Cream*

It was thought desirable to investigate separately the distribution of soluble ingredients of milk and cream during freezing, since they would be



much more likely to diffuse into the unfrozen portion than any of the others. This was first studied by determining the freezing points of the unfrozen portions of the products used previously. Determinations were made at various degrees of freezing as before. In addition, a fifth series consisting of 13-15 per cent homogenized cream was included in the comparison.

The results are shown in Figure 4. Apparently the fat phase exercises a strong restraining action on the diffusion of soluble substances into the unfrozen portions of the freezing products. This is not particularly evident in the case of 4 per cent milk but shows clearly with the 13-15 per cent cream while with the 25 per cent cream diffusion is apparently prevented entirely. Of particular interest are the results obtained with homogenized 13-15 per cent cream. The creation of a stable fat emulsion and increased viscosity in this product reduced diffusion almost as completely as in the 25 per cent cream. This again emphasizes the relation of cream rising and fat globule

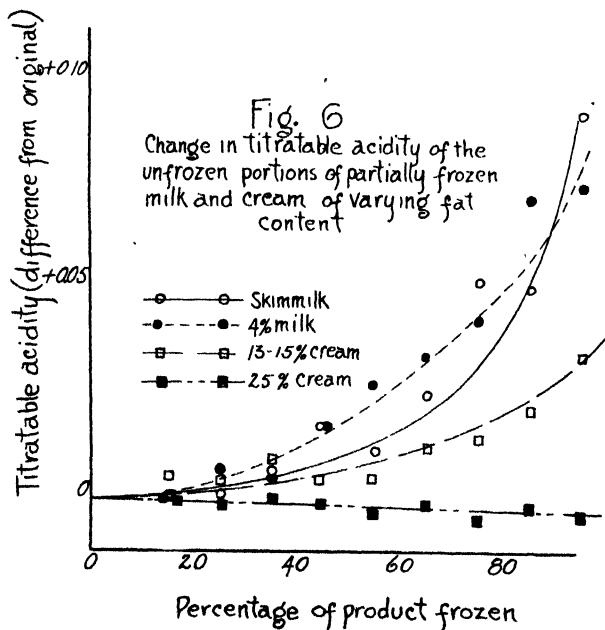
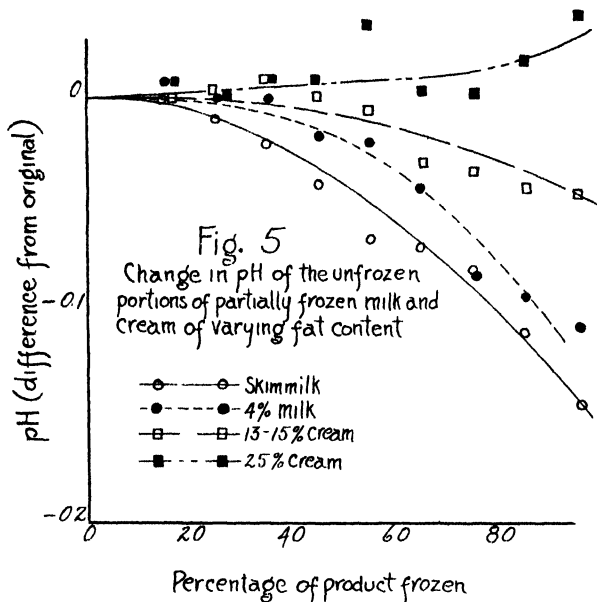


clustering to the concentration of materials in the unfrozen portions. Homogenization of the whole milk gave results (not shown) little different from those obtained without homogenization, indicating that the amount of fat is the controlling factor and the stability of the emulsion a secondary one.

Further supporting data showing the effect of the fat phase on the concentration of soluble materials in the unfrozen portions of partially frozen milk and cream are given in Figure 5 and Figure 6. Figure 5 exhibits the pH of the unfrozen portions during the course of freezing and Figure 6 the titratable acidity.

The data show conclusively that no appreciable increase in soluble substances takes place in the unfrozen portion of cream containing as much as 25 per cent of fat in the course of freezing. The data for richer cream are not included. Likewise analyses of the frozen portions of the samples are omitted since the results were what would be expected from a study of the data in Figures 4, 5 and 6.

The slight increase in pH of the unfrozen portion of 25 per cent cream as freezing progress is interesting. In a paper to follow, further data show this occurs consistently when cream is frozen or partially churned, whereas when the emulsion is completely broken, the pH of the serum drops back to about the original figure.



The decrease in titratable acidity of the unfrozen portion of 25 per cent cream as freezing progresses is so small to be significant and is probably due to the occlusion of small amounts of "acid" substances in the butter particles commonly noted in cream frozen more than 40-50 per cent.

#### SUMMARY AND CONCLUSIONS

When whole milk is partially frozen in an undisturbed condition, the fat concentration in the frozen and unfrozen portions over the entire range of freezing is dependent upon the cream rising phenomenon of the milk and its speed relative to the speed of freezing; also to some extent, naturally, on the shape and size of the container. Milk that exhibits normal creaming ability shows variable concentrations of fat in the frozen and unfrozen portions from the time freezing first becomes evident until the milk is completely frozen.

When the creaming ability of the milk is destroyed (by heating or homogenization), the fat concentration of the unfrozen portion increases progressively with the degree of freezing, while that of the frozen portion is decreased at first, but finally approaches the fat percentage of the original milk as the degree of freezing approaches 100 per cent.

Increasing fat concentrations in milk or cream retard the diffusion or concentration of the milk constituents (both colloidal and soluble) into the unfrozen portion of the freezing products, and when the fat concentration reaches 25 per cent, such diffusion is practically prevented and the cream freezes homogeneously. The diffusion is possibly inhibited because of the increased viscosity with increasing fat content and also because of the more effective sealing of the interstices between the developing ice crystals by the increased amount of fat in the form of solidified globules.

Homogenization acts similarly to increasing the fat content in restricting the concentration of ingredients in the unfrozen portions of freezing cream provided there is sufficient fat present to give an increase in viscosity.

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## VITAMIN A CONTENT OF PASTURE PLANTS

### IV. WHITE BLOSSOM SWEET CLOVER (*MELILOTUS ALBA* DESVX.), ORCHARD GRASS (*DACTYLIS GLOMERATA* L.) AND MEADOW FESCUE (*FESTUCA ELATIOR* L.) UNDER PASTURAGE CONDITIONS AND FED GREEN\*

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In the three previous papers from this station the vitamin A activity of the following six pasture plants were reported: white clover, Kentucky bluegrass, timothy, red top, alfalfa, and smooth brome. The plants reported herein are sweet clover, orchard grass, and meadow fescue. These nine pasture plants are probably more universally used in pasture mixtures than any other plants. Although alfalfa is included in the list it is not used extensively as a pasture crop. Its vitamin A activity in the fresh green state is of interest, however, for comparison with reports on the vitamin A activity of alfalfa hay.

Because of previous literature reviews, citations will be limited to those pertinent to this report. The orchard grass and meadow fescue studied were grown under irrigation at the Caldwell Substation of the Idaho Agricultural Experiment Station. In addition to second-year sweet clover under the same conditions, both first-year and second-year non-irrigated sweet clover grown at Moscow were studied. The same experimental procedure was used as in the second report (6). Fresh green samples of the plants were received at the laboratory twice a week. The samples were obtained under typical pasturage conditions. The period covered by the experimental feeding was later in the summer than the period represented in previous reports on other plants.

#### • RESULTS

The average weight of the rats in all groups was 39 grams at the beginning of the depletion period and 96 grams at the end. The depletion period for all the rats averaged 29 days.

The average growth response of the rats when fed each of the plants as a vitamin A supplement is shown in Table I and Figure 1. Results are presented for periods of four weeks (28 days) and the vitamin A values are expressed in rat units calculated from the dose in milligrams which caused a gain of 12 grams in four weeks.

\* Published with the approval of the Director as Research Paper No. 138 of the Idaho Agricultural Experiment Station.

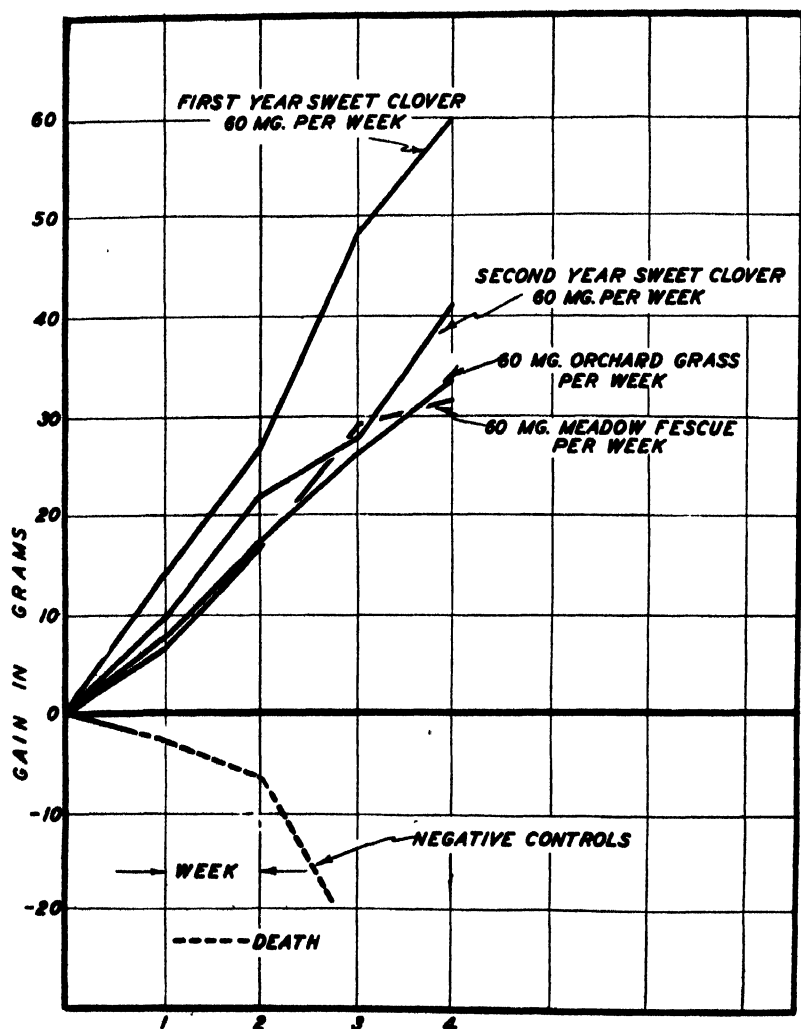


FIG. 1. Average weekly weight increases of rats, previously depleted of vitamin A, when fed either white blossom sweet clover, orchard grass, or meadow fescue as a supplement to a vitamin A free diet.

Average weekly losses in weight of negative control rats were computed each week by averaging the weight of all living rats as long as 50 per cent of the original group survived. The line was terminated by extending it directly from the last week showing 50 per cent survival to a point indicating the average survival days and average weight at death for the entire group. The broken line indicates that one or more deaths occurred during the week.

Based on the growth response obtained with other plants at various levels, each pasture plant in this report was fed at a level of 60 milligrams per week. The animals (10 rats) receiving second-year sweet clover made an average total gain in four weeks of  $29 \pm 2$  grams, which would indicate that this plant contained  $242 \pm 19$  rat units of vitamin A activity per gram. Eleven rats fed first-year sweet clover made an average gain of  $60 \pm 4$  grams, indicating at least  $500 \pm 30$  rat units of vitamin A activity. The Pharmacopoeia of the United States (3), however, prescribes that, in establishing the vitamin A potency of cod liver oil, data shall be considered valid when two-thirds of the animals in a group have made individual gains in four weeks of 12 grams or more and not in excess of 60 grams. Since 5 of the 11 rats fed first-year sweet clover gained more than 60 grams and none less than 37 grams, it would seem that definite evaluation of the vitamin A activity of this plant can not be made without additional feeding tests at lower levels. Comparison of pasture plants on the same feeding level has some advantage in attempting to establish their relative rank in vitamin A activity, and the results are presented for the first-year sweet clover group with due regard for their limitations.

Seven rats that received orchard grass as a vitamin A supplement in 1932 made an average total gain of 33 grams, while seven rats receiving the same grass in 1934 averaged 32 grams gain. The weighted average of the 14 rats in these two groups showed an average total gain of  $32 \pm 2$  grams, indicating that orchard grass contained  $275 \pm 13$  rat units of vitamin A activity per gram.

Meadow fescue was also bio-assayed for vitamin A activity during two different years, 1932 and 1934. In 1932 eight rats averaged 34 grams in total gain and in 1934 four rats averaged 26 grams. The weighted average of the 12 rats was  $30 \pm 2$  grams of total gain in four weeks. This would indicate that the meadow fescue contained  $250 \pm 13$  rat units of vitamin A activity.

The coefficient of variation in the group fed second-year sweet clover was 36 per cent, for the group fed first-year sweet clover 28 per cent, for the orchard grass group 25 per cent, and for the meadow fescue group 27 per cent. The variation in each group compares favorably with what may be expected as reported by Sherman and Burtis (4). Statistical analysis of the results showed that a true difference existed between first-year sweet clover and the other three pastures, the difference being from 11 to 15 times the probable error of the difference. The differences among the vitamin A values of orchard grass (275 rat units), meadow fescue (258), and second-year sweet clover (242), however, were not statistically significant.

In order to be able to compare the results with data reported on the basis of 24 grams gain in 8 weeks as a standard unit of growth, the average total gains for each group of rats for 8 weeks are given. Since rats which die

during the feeding period are often included in the average for a group, such rats were also included in summarizing the data, herein reported, on an 8 weeks' basis.

When the rats were fed 60 milligrams of second-year sweet clover, the total gain in 8 weeks averaged  $33 \pm 3$  grams per rat, indicating the content of vitamin A activity to be  $138 \pm 14$  rat units per gram. Rats fed the same amount of first-year sweet clover averaged  $99 \pm 6$  grams in total gain, indicating that this pasture plant contained  $413 \pm 27$  rat units of vitamin A activity per gram.

Orchard grass fed on the same level resulted in an average total gain of  $29 \pm 3$  grams per rat. This would indicate that the orchard grass contained  $121 \pm 13$  rat units of vitamin A activity per gram. Meadow fescue fed in like quantities caused an average gain of  $23 \pm 5$  grams per rat, or  $96 \pm 21$  rat units of vitamin A activity per gram for this grass.

The rats used as negative controls all showed typical symptoms of vitamin A deficiency at death. Of the 14 rats, 10 had infected sinuses, 10 had infected inner ears, 9 had infection at the base of the tongue, 5 had hemorrhagic intestines, and 5 had infected lymph glands. The negative controls survived an average of 19 days and lost an average of 19 grams in weight.

#### DISCUSSION

It is interesting to note that first-year sweet clover contained at least twice as much vitamin A activity as second-year sweet clover. This may be accounted for by the fact that first-year sweet clover is much finer stemmed and usually has a larger percentage of leaves than second-year sweet clover. Hauge (1) has reported that in alfalfa hay the vitamin A activity is primarily in the leaves.

In comparison with other pasture plants previously studied (5, 6, 7) the plants herein reported would have the following rank in rat units of vitamin A activity per gram:

First-year sweet clover	$500 \pm 30$
Smooth brome grass	$396 \pm 27$
Red top	$308 \pm 10$
Orchard grass	$275 \pm 13$
Alfalfa	$269 \pm 17$
Meadow fescue	$250 \pm 13$
Second-year sweet clover	$242 \pm 19$
White clover	$242 \pm 19$
Timothy	$220 \pm 13$
Kentucky bluegrass	$175 \pm 11$

In each of the four papers of the series statistically significant differences were found among the grasses studied. For example, red top was statis-

tically superior to timothy in paper II and smooth brome was superior to alfalfa in paper III. By chance, rather widely varying plants were paired for study in the different feeding tests. When the results from all the pasture plants were analyzed statistically, however, different deductions were made.

Considering the pasture plants in the order listed above the difference between any one plant and the one ranking next above or below it was of little, if any, statistical significance. In fact, orchard grass, alfalfa, meadow fescue, second-year sweet clover, and white clover had no significant differences from each other. Selected plants, however, do have significant differences. For example, the difference between red top and timothy is eight times the probable error of the difference.

These facts would indicate that the relative ranking of the plants is justified but that the difference between any adjoining plants as ranked in the list is not particularly significant.

Since the plants studied are among the most universally used pasture plants it would appear that pasture is an extremely potent source of vitamin A activity.

A cow can easily consume 100 pounds of pasture grass daily. If the pasture had a vitamin A value of 200 rat units per gram the total intake of vitamin A activity would be more than nine million rat units.

Moore (2) has shown that the cow's daily output of carotene and vitamin A in the milk fat is very small compared with the carotene intake, never exceeding that of normal summer butter regardless of the amount in the feeds consumed. Considering the large quantity of pasture consumed daily by the dairy cow, and the fact that all the pasture plants have high vitamin A values, it would seem that dairymen might use mixtures of any of the pasture plants adaptable to their particular climatic and soil conditions without the vitamin A activity of the pasture being a limiting factor.

#### CONCLUSIONS

Biological assay of second-year white blossom sweet clover (*Melilotus alba* Desvx.) indicated that it contained  $242 \pm 19$  rat units of vitamin A activity per gram when sampled under pasturage conditions and fed in the fresh green state. First-year white blossom sweet clover contained  $500 \pm 30$  units per gram. Under similar conditions orchard grass (*Dactylis glomerata* L.) contained  $275 \pm 13$  units, and meadow fescue (*Festuca elatior* L.)  $250 \pm 13$ .

The relative rank of these pasture plants compared with others previously reported is as follows: First-year sweet clover,  $500 \pm 30$  rat units per gram; smooth brome,  $396 \pm 27$ ; red top,  $308 \pm 10$ ; orchard grass,  $275 \pm 13$ ; alfalfa,  $269 \pm 17$ ; meadow fescue,  $250 \pm 13$ ; second-year sweet clover and

Average Growth Response of Rats when Fed Fresh White Blossom Sweet Clover, Orchard Grass, and Meadow Fescue

PASTURE PLANT	SOURCE	DATE OF TEST PERIOD	AMT. OF PLANT FED WEEKLY	RATS FED		RATS USED		AVE. WT. AT BEGINNING OF DEPLETION PERIOD		AVE. WT. AT END OF TEST PERIOD		AVE. GAIN IN WT. IN 4 WEEKS		CORRELATION OF VARIATION	RAT UNITS OF VITAMIN A
				No.	%	No.	%	Grams	Grams	Grams	Grams	Grams	Grams		
(2nd yr.) White Blossom Sweet Clover	Caldwell	7-9-32 to 9-3-32	0.060	8	100	8	100	42	100	131	31				
"	Moscow	6-3-34 to 7-29-34	0.060	3	67	2		41	93	116	23				
Average				11	91	10		42	99	128	29 ± 2			36	242 ± 19
1st yr.) Sweet Clover	Moscow	7-23-34 to 9-17-34	0.060	11	100	11		41	95	155	60 ± 4			28	500 ± 30
Orchard Grass	Caldwell	7-9-32 to 9-3-32	0.060	8	88	7		39	97	130	33				
"	"	5-30-34 to 7-25-34	0.060	7	100	7		37	97	129	32				
Average				15	93	14		38	97	130	33 ± 2			25	275 ± 13
Meadow Fescue	Caldwell	7-9-32 to 9-3-32	0.060	8	100	8		39	99	133	34				
"	"	5-30-34 to 7-25-34	0.060	5	100	5		38	103	129	26				
Average				13	100	13		39	101	131	30 ± 2			27	250 ± 13
Negative				14				39	92	Average survival—19 days Ave. loss in wt.—19 grams					

white clover,  $242 \pm 19$ ; timothy,  $220 \pm 13$ ; and Kentucky bluegrass,  $175 \pm 11$ .

Although the differences among the higher ranking plants and the lower ranking ones were statistically significant, little, if any, significant difference existed between any one plant and the plant listed next above or below it.

The pasture plants studied are among the most common plants used in pasture mixtures. The fact that they were all found to have high vitamin A values would seem to justify the conclusion that pasture is a potent source of vitamin A activity.

Considering the large quantity of pasture consumed daily by the dairy cow, and the fact that all the pasture plants have high vitamin A values, it would seem that dairymen might use mixtures of any of the pasture plants adaptable to their particular climatic and soil conditions without the vitamin A activity of the pasture being a limiting factor.

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# BACTERIOLOGICAL METHODS FOR THE ANALYSIS OF DAIRY PRODUCTS

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## FOREWORD

"In 1927, the Committee on Bacteriological Methods of the American Dairy Science Association began a series of reports dealing with the various laboratory procedures suitable for the bacteriological analysis of dairy products. This committee has functioned through the agency of subcommittees, composed of those who are especially interested in certain phases of the dairy industry."

"The objective of these committees has been to make available in condensed form various methods for the analysis of dairy products. The views expressed in these reports are those of Committees appointed by the American Dairy Science Association. As such they are printed for the criticism of other members of the Association and other interested persons."\*

## THE BACTERIOLOGICAL EXAMINATION OF EVAPORATED MILK

### (Sterilized)

Evaporated milk, as found upon the market, is primarily confined to cans in which the product has been subjected to sterilization by heat. The usual bacterial content of such milk is relatively low and for that reason the bacteriological study is somewhat different than the study of some of the related products.

## METHODS OF SAMPLING

It is extremely important in the sampling of this product, especially if it is taken from the original can, that no contamination occur during handling and that the sample container be sterile. All the utensils that are used or in any way come in contact with the product must be sterile, and the room in which the work is carried out should be as free as possible from atmospheric contamination. This is necessary because of the almost sterile condition of the normal product when in the container.

A very suitable method of handling canned milk previous to sampling is to shake the can and its contents thoroughly, clean the outside of the can, wipe dry with a clean towel and flame the surface to be punctured. In cases where it is desirable to seal the can after removing the sample, the surface

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\* See JOURNAL OF DAIRY SCIENCE 16: 277. 1933.

can be tinned with soldering iron before being punctured. This procedure will materially aid in the sealing process. The can-opening appliance should be sterilized in the flame before using. The opening should be only large enough to permit the insertion of a pipette (usually 1 ml.) for the removal of a suitable quantity of the contents, and this should be done as rapidly as possible to eliminate possible contamination from the air. The opening may be protected by covering the entire can with a sterile petri plate half.

If further work is to be carried out with the sample, the contents of the container may be transferred to a glass-stoppered sterile bottle and stored at refrigeration temperatures. The glassware, sampling tubes, etc., should be sterilized in accordance with "Standard Methods of Milk Analysis." If samples are collected in the plant, great care should be taken to see that the product is not in any way contaminated by the movement of the valves on the receptacle from which the sample is drawn. A slight movement of such a valve will often dislodge large numbers of organisms which will be washed out by the product, giving erroneous results. This can be overcome by sampling directly from the large container with some suitable sampling device or if it is absolutely necessary to obtain the sampling through the outlet, it should only be taken after the product has flowed continuously for some few minutes. Either of these methods will help to eliminate inaccurate samples.

If it is necessary to prepare a composite sample it may be secured by combining the various batches, collecting samples as outlined in a container of such a size that it will permit thorough mixing of the product at the time of the analysis.

If the sample of evaporated milk completely fills the container in which it is received, the entire contents should be transferred to another clean, dry, sterile container of ample size to permit proper mixing by shaking. The necessity of uniformity in the sample should be understood by all persons interested in bacteriological work, but the detailed manipulations may be left to the discretion of the individual. It is important during the handling of the products that all possible contamination from a dusty atmosphere or unsterile utensils be prevented. The same general precautions as are applied to the preparation of any sample for bacteriological analysis apply to the product.

#### SUGGESTED METHODS OF EXAMINATIONS

##### (a) *Standard Agar Plate Method*

The standard agar plate method (1) of the American Public Health Association, as used for fluid milk, may be used for the examination of evaporated milk.

*Media:*

Standard nutrient agar prepared according to "Standard Methods of Milk Analysis" (1) may be used.

For special research and investigational work and also routine work involving a study of the possible sterility of the product, it is suggested that beef infusion agar be used. For directions for preparation see Methods for the Microbiological Analysis of Butter (2).

*Preparing Dilutions:*

It is believed desirable to follow as closely as practical, methods already recognized as standard procedures. For that reason the method of bringing the volume or weight up to 100, as used in the Standard Methods of Milk Analysis of the American Public Health Association (1), is preferred to the method of adding the material to be tested to 10 ml. or grams as the case may be. It is recommended that for normal evaporated milk the volumetric method be used, transferring 1 ml. or 10 ml. of the product to the 99 ml. or 90 ml. water blanks, respectively. Samples that are abnormal physically, such as "curdled" or "lumpy," are best brought to dilution by the gravimetric method.

For direct plating of a low-count milk, one ml. or gram of the sample is placed directly in the petri plate. For a one to ten dilution, it is preferred to measure 10 ml. or weigh 10 grams of the evaporated milk directly into the dilution bottle containing 90 ml. of sterile distilled water. If higher dilutions are desired, they may be prepared from the one to ten dilution or one ml. or gram of the product may be used in 99 ml. of water to obtain a 1:100 dilution. The practice of adding the product to water blanks instead of the reverse is suggested because of danger of contamination in transferring a large volume of water; this is important with a low-count or sterile product. Plating should be completed within 15 or 20 minutes after the first dilution in order to prevent growth or death in the dilution bottle which would affect the final results.

In the case of coagulated samples, the use of N/10 LiOH (4) as the diluent in order to aid in getting the product into better solution is advised. In the case of such samples, the diluted product should be kept thoroughly agitated just prior to withdrawal of the sample with the pipette in order that a uniform sample of the solution may be transferred to the petri plate. This condition is most likely to occur in the study of abnormal samples of evaporated milk.

*Preparing Plates:*

The pouring of the plates should follow the suggestions outlined in the latest edition of the Standard Methods of Milk Analysis (1). With normal evaporated milk, direct plating of the product (1 ml. per plate) will yield either no colonies, or at best, very few. Hence the usual selection of dilu-

tions to yield from 30 to 300 colonies is not possible. However, at least two dilutions should be plated in every case, with duplicate plates for each dilution.

#### *Incubation:*

For standard results with standard nutrient agar, use an incubation period of 2 days at 37° C. as recommended in Standard Methods of Milk Analysis (1). Other incubation periods and temperatures may be used for special purposes, such as 5 days at 25° C., 3 days at 30° C., or 2 days at 55° C. The latter temperature is recommended for estimating the number of thermophilic bacteria which may be present.

#### *Counting of Plates:*

In the case of those samples which are abnormal and contain material which fails to go into suspension readily, extreme caution should be exercised in counting the incubated plates in order to differentiate between bacterial colonies and masses of the constituents of the samples. Devices for counting the plates with the aid of artificial illumination and low magnification are strongly recommended. Doubtful objects should be examined with higher magnification to determine whether or not they are colonies.

#### *(b) Test for Sterility*

For the purpose of determining whether or not the product in the cans is sterile, it is recommended that sample cans be held at 37° C. for one week. At the expiration of the period the cans should be plated as described above. Cans in which an abnormal physical condition of the milk has developed may have large numbers of organisms present and should be plated in high dilutions. Cans which show the milk in normal condition and which yield no colonies on any of the plates, including 1 ml. direct, may be assumed to be, for all practical purposes, sterile.

#### *(c) Direct Microscopic Count (Breed Method)*

The direct microscopic count (1), as applied to milk, would normally be of little value for evaporated milk because of the low bacterial content of the product, however, in the study of cases of spoilage the method is of value. If it is used for this purpose, it should be carried out according to directions given by Fay (3) for ice cream.

#### *(d) Anaerobic Agar Plate Method*

In the bacteriological study of evaporated milk it is often desirable to obtain information regarding the numbers of anaerobic organisms present in the sample. If this is desired, the same procedure as outlined under "Standard Agar Plate Method" may be carried out, except for the addition of 1 per cent of dextrose to the standard nutrient agar. The methods of

incubation must necessarily be carried out as described in text books dealing with the study of anaerobic bacteria. This method will be of special value in the study of abnormal products and may find a place in certain routine work.

(e) *Examination for Thermophilic Bacteria*

For the determination of the presence of thermophilic organisms plates may be prepared from standard nutrient agar. For investigation or research purposes, it is recommended that the following materials be added to each 1,000 ml. of standard agar.

Bacto Tryptophane Broth	2.5 grams
Bacto Yeast Extract	1.0 gram
Dextrose	1.0 gram
Reaction	pH 7.0 $\pm$ 0.2

The plates should be incubated for 48 hours at 55° C. At least 15 ml. of medium should be used in each plate to prevent excessive drying during the incubation. Water should be placed in the incubator in order to reduce the drying of the plates to a minimum.

(f) *Examination for Spore-Forming Bacteria*

The sample of the product should be placed in a sterile container, heated in a water bath to 80° C. and held for 10 minutes; after cooling, the product should be plated by the standard plate method direct from the sample. Such plates should be incubated 3 days at 37° C. to allow development of such types as *B. coagulans*.

(g) *Medium for Special Purposes*

The medium outlined in the standard procedure of the American Public Health Association (1) may not be conducive to the growth of all organisms in average milk. If a special medium is desired, the following is suggested:

Bacto Tryptophane Broth	2.5 grams
Bacto Peptone	2.0 grams
Bacto Yeast Extract	1.0 gram
Dextrose	5.0 grams
Agar	15.0 grams
Water	1000 ml.
Reaction	pH 6.8

Incubation with this medium may be 3 days at 30° C. or any other time or temperature in accordance with the results desired.

METHOD OF REPORTING COUNTS

Results on normal evaporated milk should be reported per milliliter of the product. When the condition of the milk necessitates weighing, results

should be reported per gram. In order to have uniformity in reporting, the medium used and the time and temperature of incubation should always be given in direct conjunction with the numerical results obtained. It is desirable that this procedure be followed when the standard medium and incubation are used as well as in those cases wherein special media and variable incubation conditions are employed.

#### THE BACTERIOLOGICAL EXAMINATION OF CONDENSED MILK

##### *(Incompletely Sterilized)*

Under this heading will be included those products that may or may not contain added sugar but which have not been sterilized in the container. The most common of these products is the sweetened condensed milk in hermetically sealed cans. However, much routine bacteriological work is carried out in the examination of sweetened or unsweetened products in bulk containers, such as ten-gallon cans and barrels.

#### METHOD OF SAMPLING

The same procedure as outlined for evaporated milk may be carried out in sampling products enclosed in hermetically sealed cans. A difference will be found, however, in the type of product. The sweetened condensed milk may be found to be extremely thick and viscous. In such cases it may be advisable to place the can of product in a warm water bath at a temperature not over 37.5° C. for a short time so that the product will become less viscous. In some cases where the separation of sugar has been unusually heavy, it may be necessary to transfer the entire contents of the container to another sterile receptacle to insure a proper mixing. When samples are to be taken from barrels the same precautions should be exercised as outlined above except that it will be impossible to mix the contents. The danger of contaminating the sample when being drawn from the storage tanks should not be overlooked as the movement of valves or pipe joints may dislodge large numbers of organisms which will contaminate the sample and give inaccurate results. If samples must be taken from large masses of the product, they may be obtained by the use of sterile tubes, dippers, or other suitable equipment. Care should be taken that the sample is obtained below the surface which has been exposed to the atmosphere.

If samples are to be taken from barrels they may be obtained either by drawing the sample from the bottom of the barrel or, when taken after the head of the barrel is completely removed, they should be obtained below the surface by some suitable sampling apparatus.

Where it is necessary that composite samples be taken, the final sample should be in a container sufficiently large to allow mixing. Very satisfactory containers for this purpose are glass-stoppered bottles of suitable size.

## PREPARING THE SAMPLE FOR EXAMINATION

If the sample of condensed milk completely fills the container in which it is collected or received, the entire contents should be transferred to another clean, dry, sterile container. The detailed manipulations of the process are left to the discretion of the individual. The same general precautions that are applied to any sample for bacteriological analysis should be applied to this product.

## SUGGESTED METHODS OF EXAMINATION

*(a) Standard Agar Plate Method*

The standard agar plate method (1) as used for fluid milk may be used for determining the numbers of organisms in condensed milk.

*Media:*

Standard nutrient agar prepared according to "Standard Methods of Milk Analysis" (1) may be used.

For special research and investigational work and also routine work in which maximum counts are desired, it is suggested that beef infusion agar be used. For directions for preparation see Methods for the Microbiological Analysis of Butter (2). The addition of one per cent of sucrose may be desirable in some types of work.

*Preparing Dilutions:*

For plain condensed (unsweetened) milks it is recommended that the volumetric method be used for the preparation of dilutions. The thicker, sweetened products must be weighed. For the 1:10 dilution, measure 10 ml. or weigh 10 grams of the condensed milk into a dilution bottle. In some instances it may be more convenient to prepare the 1:10 dilution by using 45 ml. of water and 5 ml. or 5 grams of condensed milk. If a 1:100 dilution is desired, this may be prepared from the 1:10 dilution, or one ml. or one gram of condensed milk may be diluted with 99 ml. of distilled water. The dilutions may be conveniently prepared by weighing the condensed milk directly into a bottle before adding the dilution water, or if desired, the milk may be weighed into the bottle containing the dilution water. The plating should be completed within 15 to 20 minutes after the addition of water in order to prevent growth or death in the dilution bottle which would affect the final results.

In the case of abnormally thickened milk, it is often desirable to use sterile pieces of broken glass or glass beads in the dilution bottle to assist in the mixing of the sample in the dilution water. In such products the use of N/10 LiOH (4) as the diluent may aid in dissolving the coagulated material.

*Preparing Plates:* (see page 649)

*Incubation:* (see page 650)

*Counting of Plates:* (see page 650)

(b) *Microscopic Colony Count (Frost Method)*

In laboratories already equipped and experienced in counting bacteria in milk by this method, it would seem that this method could be readily used in the routine control of condensed milk. Directions as outlined in the Standard Methods of Milk Analysis (1) may be followed.

The original sample should be diluted as suggested in the methods for the agar plate count. Incubation should be for 12 to 16 hours at 37° C.

(c) *Direct Microscopic Count (Breed Method)*

The direct microscopic count (1), as applied to milk, may be of value in the study of condensed milks, especially in unsweetened condensed milks. Where this method is used it should be kept in mind that it cannot be relied upon as an adequate procedure for the determination of bacterial count, but it may be used to give valuable information regarding the product. Where this method is used it should be carried out according to the directions given in the Standard Methods of Milk Analysis (1) or as outlined by Fay (3) for ice cream. It may be of supplementary value in conjunction with certain procedures listed herein.

(d) *Examination for Hemolytic Streptococci*

The examination of certain types of condensed milk for hemolytic streptococci may be of value, especially for milk made without the usual hot-well treatment. For this examination, standard nutrient agar to which has been added 0.5 per cent sodium chloride and 2 to 5 per cent defibrinated blood is suggested for plating. The blood should be added aseptically after the agar is melted and cooled prior to pouring the plates. The plates should be incubated for 2 days at 37° C. Examination should be made at the end of 24 and 48 hours.

Suspicious hemolytic colonies should be examined by Gram staining to determine the presence of streptococci. Before definite conclusions can be drawn, reference should be made to standard text-books on bacteriology dealing with the recognition and differentiation of hemolytic streptococci.

If conditions permit its use, veal infusion medium will give somewhat better results than the standard nutrient agar for the growth of the hemolytic streptococci. This medium is prepared as follows:

Ground lean veal	500 grams.
Distilled water	1000 ml.

Infuse overnight in a cold room. Strain through cheese cloth by pressing. Make up to the original volume. Bring to boiling with frequent stirring and boil until the infusion is clear and the coagulum brown. Strain through cheese cloth and filter through paper. Adjust reaction to pH 6.8 to 7.0. Add 0.5 per cent Difco peptone and 0.5 per cent sodium chloride. The broth should then be heated in the autoclave for 15 minutes at 20 pounds pressure. This is a higher pressure than that used for the final sterilization and is

employed to avoid precipitates in the finished product. Egg may be added previous to autoclaving if a glass-clear medium is desired. Before the final sterilization, 1.5 per cent agar is added and the medium distributed in flasks. Filter and sterilize at 15 pounds pressure for 15 minutes. Defibrinated blood (rabbit, horse, sheep, etc.) may be added aseptically just prior to plating and after the agar has been melted and cooled to 50° C.

(e) *Examination for Thermophilic Bacteria* (see page 651)

(f) *Examination for Bacteria Causing Thickening*

For the determination of the presence of bacteria capable of causing thickening in sweetened condensed milk, the following procedure may be followed: The sample of sweetened condensed milk in question should be diluted with sterile water in a glass-stoppered bottle so that the concentration of sucrose in water in the sample will be approximately 40 per cent. For ordinary purposes approximately 90 grams of milk and 10 grams of water will be sufficient. After the sample has been thoroughly mixed by shaking it should be incubated at 30° C. for a period of one month. If organisms capable of causing bacterial thickening are present the diluted milk will become thick and the titratable acidity materially increased. Cultures of the thickening organisms can readily be obtained by plating on nutrient agar and incubating for 2 days at 37° C.

(g) *Examination for Gas-Forming Organisms*

For the determination of gas-forming organisms the inoculation of one ml. of the milk into deep agar of the composition indicated on page 651. A layer of sterile agar may also be placed on top of the hardened inoculated agar and the tube incubated at 37° C. for 48 hours.

*Tests for Yeasts Capable of Causing "Swells"*

Sucrose-fermenting yeasts capable of growth and gas production in high concentrations of sucrose are always a potential source of trouble in the manufacture of sweetened condensed milk. As a routine check for the detection of possible contamination with these types, it is recommended that sample cans be incubated at 37° C. for extended periods. If "swells" develop the milk should be plated on standard nutrient agar (1) (or beef infusion agar) to which 30 per cent sucrose has been added before sterilization.

As a further check on the condition of sweetened condensed milk immediately after manufacture, it is suggested that enrichment in 30 per cent sucrose bouillon can be used, noting the presence or absence of gas production after incubation for 2 days at 37° C., followed by plating on the sucrose agar. The presence of yeasts capable of fermenting this concentration of sucrose with gas production is the signal for a careful study of sources of contamination in the plant, which must include taking samples at various stages in the manufacture and their subjection to the same test. Whether

or not the types isolated are capable of causing "swells" must be determined by direct inoculation into cans of fresh product.

The direct microscopic count on both the fresh product and that which has been incubated for enrichment purposes may be useful in the examination for yeasts. This would of necessity be supplemented by cultural methods to determine the types of yeasts present.

#### (h) *Examination for Yeasts and Molds*

The determination of yeast and mold counts in condensed milk, while not a common practice, can be used quite satisfactorily and should give valuable information. No doubt a more complete study of yeasts and mold content of condensed milk would give much information regarding the sanitary conditions under which the product was produced. For details of the determination see *Methods for the Microbiological Analysis of Butter* (2).

#### (i) *Medium for Special Purposes* (see page 651)

#### METHOD OF REPORTING COUNTS

The results should normally be reported per milliliter or gram of product. One variation from standard methods will no doubt have to be included in the study of condensed milk inasmuch as many of the plates will show less than 30 colonies. In this case it is recommended that the actual number of colonies be used as an indication of the count.

In the interests of uniformity of practice and interpretation of data from various sources, the medium used and the time and temperature of incubation should always be reported in direct conjunction with the numerical results obtained. It is desirable that this procedure be followed when the standard medium and incubation are used as well as in those cases wherein special media and variable incubation conditions are used.

#### CONCLUSION

The views expressed in this report are those of a committee appointed by the American Dairy Science Association. As such they are submitted for the general criticism of members of the American Dairy Science Association and other interested parties.

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# AN UNNOTED HEMOLYTIC STREPTOCOCCUS ASSOCIATED WITH MILK PRODUCTS

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Dick and Dick (5) have reported the presence in small numbers of beta hemolytic streptococci in some of the samples of commercial infant food preparations, made from milk, which they have examined. While these workers made no such statement, it was at first inferred by some that these streptococci might have been the cause of an outbreak of gastro-intestinal trouble in a home for children, but this view was not supported by later work (Dick, Dick and Williams, 6). That hemolytic streptococci are not generally present in significant numbers in dried milks and infant foods containing milk powder is indicated by the surveys of Hucker and Hucker (7) and Supplee and Bixby (12), in which a variety of such products was examined.

Through the cooperation of a manufacturer we obtained a few samples of powdered milk and "protein milk" which contained beta hemolytic streptococci, one of these samples coming from the same factory batch which was examined by the Dicks. The presence of hemolytic streptococci in these products is somewhat surprising, since in their manufacture the milk is first pasteurized and then subjected to the drying process at relatively high temperatures.

On detailed study these organisms were found to possess characteristics which would appear to justify the conclusion that they represent a type of streptococcus not heretofore described. Forty cultures were studied, but since they were all isolated from the products of one factory it is likely that they represent closely related strains of one type.

## GENERAL CHARACTERISTICS

The morphological, cultural, and staining properties of this organism are those characteristic of the streptococci and need not be detailed here. Occurrence of the cells in pairs and in short and long chains is somewhat variable on different media and varying conditions of growth, as is true of the streptococci in general.

### *Action on blood*

Active hemolysis of the beta type is produced on horse, human, and rabbit bloods. After five years' culture in laboratory media without blood, it was still hemolytic.

### *Action on litmus milk*

Milk is acidulated and curdled. Litmus in milk is not reduced prior to curdling; slow and incomplete reduction takes place after curdling.

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### *Action on gelatin and casein*

It does not liquefy gelatin, nor is there any visible digestion of casein in milk.

### *Temperature limits of growth*

Growth takes place at 10° C. and at 45° C. The maximum temperature for growth is about 50° C. These facts are important, since none of the pathogenic types of hemolytic streptococci, so far as present information goes, is able to grow at 10° C. (Sherman and Albus, 10; Ayers and Mudge, 2). While the maximum growth temperatures of pathogenic streptococci have not been thoroughly studied, none of them has been reported able to grow at 45° C. This was shown by Sherman and Albus (10) for the types obtained from bovine udders, and by Orla-Jensen (9) with a number of pathogenic streptococci from animal and human sources.

Studies of the temperature growth limits of pathogenic streptococci have been sadly neglected. However, repeated tests in this laboratory with a relatively few authentic strains of *Streptococcus pyogenes*, *Streptococcus epidemicus*, and *Streptococcus scarlatinæ* have shown that they do not grow at 10° C. nor at 45° C.

In this connection it should be mentioned that the fecal hemolytic *Streptococcus zymogenes* has been rarely found associated with infections, but is scarcely to be considered a pathogenic species in the usual sense. It grows at 10° C. and at 45° C., and in other characteristics is closely related to the fecalis-liquefaciens groups of streptococci.

### *Thermal death rates*

All of the cultures survived a temperature of 62.8° C. (145° F.) for thirty minutes in skimmed milk, while a majority of them withstood thirty minutes at 65.6° C. (150° F.). Since all of the human pathogenic streptococci, as well as those from bovine udders, thus far studied, are killed by exposure to 60° C. (140° F.) for thirty minutes (Ayers, Johnson and Davis, 1, and other investigators), the high thermal resistance of the organism here described is significant.

### *Limiting hydrogen-ion concentration*

In glucose broth pH values of 4.4 to 4.0 are attained. This is in marked contrast to the human hemolytic streptococci which are inhibited in a pH zone between 6.0 and 5.0 (Ayers, Johnson and Davis, 1, and many subsequent investigators).

### *Fermentation of test substances*

The outstanding feature of this organism as revealed by fermentation tests is its failure to ferment sucrose. Only one of the forty cultures was able to ferment this sugar, and that culture caused only a feeble fermenta-

tion. From the recorded literature it appears that in general the beta hemolytic streptococci ferment sucrose.

All of the cultures fermented glucose, maltose, and lactose; none fermented raffinose, inulin, nor glycerol; only five of the forty strains fermented mannitol, while 37 fermented salicin.

#### *Hydrolysis of sodium hippurate*

Sodium hippurate is fermented with the production of benzoic acid and glycine. This test, which was introduced by Ayers and Rupp (3), has been found of especial value in the differentiation of hemolytic streptococci of human and bovine origin. While not to be considered infallible, it is perhaps the best single test for this purpose (Brown, Frost and Shaw, 4). Based upon our present knowledge of the streptococci, the fact that this organism ferments sodium hippurate would appear to add strength to our belief that it is not of importance from the standpoint of human health.

#### *Pathogenicity*

The organism is not pathogenic for mice or guinea pigs by intraperitoneal injection, nor for rabbits by intravenous injection. Four mice, two guinea pigs, and two rabbits were each inoculated with one cc. of a heavy 24-hour growth of one of the strains. All of the animals remained in perfect health, and when killed three weeks later for autopsy, no lesions were observed and no organisms were recovered in cultures made from the heart, liver, kidney, and spleen. Virulence tests were not conducted with all of the forty strains.

#### *Relation to other species*

This organism, for which the name *Streptococcus hemothermophilus* is suggested, differs markedly from the known pathogenic hemolytic streptococci in its minimum and maximum temperatures of growth, thermal death point, limiting pH of growth, failure to ferment sucrose, non-virulence for laboratory animals, and (from human types) in its action on sodium hippurate.

While this organism resembles the hemolytic *Streptococcus zymogenes* in its temperature limits of growth, the two species are sharply differentiated by their respective actions on gelatin, casein, litmus milk, sucrose, raffinose, and glycerol (MacCallum and Hastings, 8; Sherman and Stark, 11).

*Streptococcus hemothermophilus* may be easily differentiated from all of the well-described species of non-hemolytic streptococci, in each case (with one exception) by four or more characteristics. According to the tests conventionally used in the study of streptococci, it is most closely related to *Streptococcus fecalis*. These two types are, however, totally different in

their actions on blood agar and their reducing abilities in litmus milk, and usually differ in their actions on mannitol.

#### ADDENDUM

As this work was done some years ago, some of the newer methods of studying streptococci, such as fibrinolytic action and the fermentation of trehalose and sorbitol, were not used. However, since these tests are of value in differentiating human and animal hemolytic streptococci which fail to hydrolyze sodium hippurate and do not reach a low limiting pH in glucose broth, they would not appear to have any obvious pertinence in this connection at the present time.

#### SUMMARY

A hemolytic streptococcus which is believed to represent a new species is described. This organism differs from the pathogenic species of hemolytic streptococci in its higher maximum temperature of growth, a lower minimum temperature of growth, a higher thermal death point, and a more acid limiting pH of growth. It also differs from the human types in the hydrolysis of sodium hippurate and its failure to ferment sucrose.

*Streptococcus hemothermophilus* (n. sp.) is suggested as a name.

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## THE EFFECT OF SOYBEANS IN THE RATIONS OF DAIRY COWS UPON THE VITAMIN A VALUE OF BUTTER\*

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It is well known that the vitamin A value of milk and butter is dependent upon the feeds fed the cow. In an earlier publication (1), it was shown that it is possible to maintain through the winter period, by practical feeding methods, the production of butter with the same vitamin A value as that produced by the cows when fed on pasture. Although good quality alfalfa hay was found to be effective in maintaining the quality of winter produced butter equal to that of pasture butter, soybean hay was somewhat less effective. No explanation was offered for the slightly lower efficiency of soybean hay as compared with pasture and alfalfa.

Since the soybean hay, which was used in the above experiment was prepared from soybean plants of excellent quality in the medium bean stage, there existed the possibility that the low vitamin A value of the butter produced by cows fed soybean hay, might be attributed to the presence of the soybeans in the hay. There is evidence that soybeans contain materials which may be of toxic nature and whose detrimental effects are eliminated or destroyed by heat. Thus, Osborne and Mendel (2) found cooking improved the feeding value of soybeans for growing rats. Robinson (3) found that hogs responded much more satisfactorily to cooked soybeans than to raw soybeans as a supplement to corn. Shrewsbury, Vestal and Hauge (4) reported that roasted soybeans have a definitely superior nutritive value to raw soybeans for both hogs and rats. Contrary to the response by hogs and rats, Hilton, Wilbur and Hauge (5) found that raw soybeans as a protein supplement to corn, oats and alfalfa hay can be utilized by dairy calves for satisfactory growth.

It therefore seemed desirable to determine what effect, if any, the beans in soybean hay fed to cows might have on the vitamin A value of butter. In order to study this factor, alfalfa hay formed the dry roughage for one group of cows and late cut soybean hay for the second group, while raw soybeans and roasted soybeans were substituted for linseed oilmeal in the grain ration of each group during successive feeding periods. Since it had been found that roasting the soybeans improved their nutritive value for growing rats and hogs, this offered a possibility that roasted soybeans might prove superior to raw soybeans in producing a butter of high vitamin A value.

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\* Published with the approval of the Director of the Purdue University Agricultural Experiment Station.

## EXPERIMENTAL

Four cows of the Jersey and Guernsey breeds were used in this feeding experiment. These cows were divided into two groups of two cows each and fed according to the schedule shown in Table I. Since it has been previously

TABLE I  
*Showing the Composition of the Rations Fed the Cows during the Successive Feeding Periods*

	LOT 1		LOT 2	
	Roughage	Grain mixture	Roughage	Grain mixture
Period 1 (6 weeks)	Alfalfa Hay Corn Silage (Check Ration)	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. linseed oil meal	Soybean Hay Corn Silage	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. linseed oil meal
Period 2 (6 weeks)	Alfalfa Hay Corn Silage	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. roasted soybeans (ground)	Soybean Hay Corn Silage	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. roasted soybeans (ground)
Period 3 (6 weeks)	Alfalfa Hay Corn Silage	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. raw soy- beans (ground)	Soybean Hay Corn Silage	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. raw soybeans (ground)

shown (1) that alfalfa hay and a balanced grain ration with linseed oil meal as the protein supplement will produce butter of high vitamin value, this ration was used as a check in these feeding trials. All cows were in their third or fourth month of lactation. The alfalfa hay fed to Lot 1 was from second cutting, of excellent quality and field cured in the absence of rain. The soybean hay fed to Lot 2 was of excellent quality. This hay was also field cured in the absence of rain. The soybeans fed in the grain rations of both groups in Period 2 were roasted in a special machine at a temperature of 350° F. for a period of 20 minutes.

At the end of each feeding period, composite samples of milk (equal amounts from each cow) from each lot were collected, the cream separated and made into butter. The butter samples were kept in cold storage at 0° F. and portions removed as needed for biological assays.

The vitamin A values of these samples of butter were determined by biological assay, using the method as previously described (1). The results of

these tests are reported in Sherman and Munsell (6) units, a unit being the amount of butter necessary to give a growth response of approximately three grams per week for eight weeks. The results of these assays are given in Chart 1.

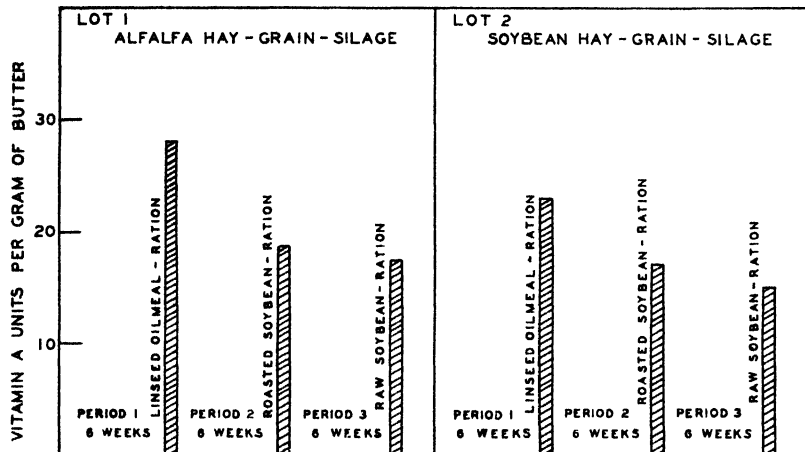


CHART 1.

Showing the suppressing effect of soybeans in the rations of cows upon the Vitamin A value of the butter.

In these experiments, alfalfa hay was the chief source of the vitamin A potency in the rations fed the cows of Lot 1 while soybean hay furnished the vitamin A in Lot 2. The cows of each lot received the same amount of these respective hays each day throughout the successive feeding periods, thus assuring a fairly constant intake of vitamin A, so that any variation in the vitamin A value of the butters produced by these cows during these periods could only be attributed to differences produced by the grain rations. In comparing the biological assays of the butters produced by the cows in Lot 1 which received alfalfa hay as the chief source of roughage, it is to be noted that the substitution of raw soybeans for linseed oil meal in the grain ration resulted in the production of butter with lower vitamin A value. Since the same amount of vitamin A was available to the cows during both periods, the raw soybeans apparently interfered in some manner with the transfer of vitamin A potency from the ration to the butter which resulted in the suppression of the vitamin A value of the butter. The attempt to eliminate the factor responsible for this suppressing action of raw soybeans by roasting the beans, gave such a small increase that it was not considered to be successful. Similar results were obtained with Lot 2.

The suppressing effect of feeding soybeans on the vitamin A content of butter is further emphasized by the relatively lower vitamin A value of the

butters produced by the cows receiving soybean hay (Lot 2) as compared to the cows receiving alfalfa hay (Lot 1). This is particularly noticeable in the first period of each lot where linseed oil meal was used in the grain ration. Apparently the soybeans in the soybean hay were responsible for the marked inhibiting action on the formation of vitamin A in the butter produced by Lot 2. These results are in accordance with our previous work (1).

From the data presented on the vitamin A value of the butter samples produced by the cows fed soybeans, it might appear that the same substance which is apparently toxic to the growth of rats may have been transmitted from the soybeans to the butter. However, this was not the case, because as is shown in Chart 2, the rats gave greater growth response with each cor-

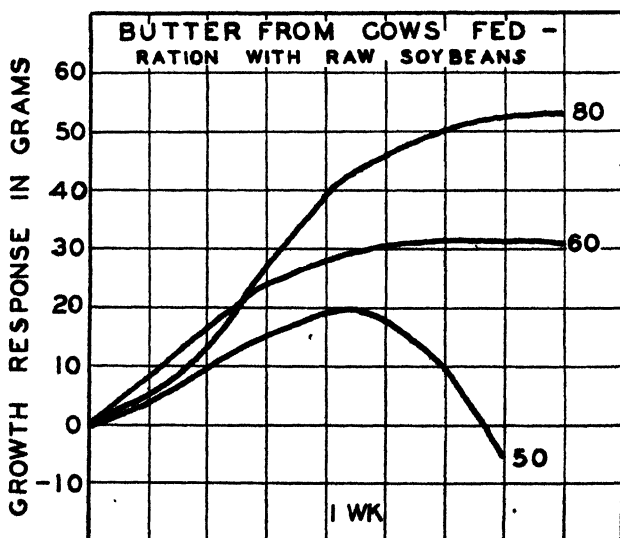


CHART 2.

Composite growth curves of rats showing that there is apparently nothing toxic transferred to the butters produced by cows receiving soybeans in the grain ration.

responding increase in the amount of butter fed. Had there been anything toxic in these samples the higher levels of feeding should have resulted in at least a partial growth failure. Furthermore, the fact that roasting the soybeans, a treatment which improves the nutritive value of soybeans for growth (4), did not eliminate the factor which suppresses the vitamin A value of butter, seems to indicate that the factor responsible for the suppression of the vitamin A value in butter is not the same as that responsible for the repressed growth response in rats.

It should be pointed out that although the feeding of soybeans in grain rations of dairy cattle does have a suppressing effect on the vitamin A forma-

tion in the butterfat, yet butters produced on rations containing soybeans are relatively high in vitamin A value as compared with butter produced by cows fed timothy hay (1) or other roughages of low vitamin A content.

#### SUMMARY

1. Dairy cows with a constant level of vitamin A intake, produced butter of lower vitamin A value when soybeans were fed in the grain ration than when linseed oil meal was used.

2. Soybeans apparently suppress the transference of vitamin A from the ration to the butter.

3. The attempt to correct this suppressing action by roasting the soybeans used in the grain ration was unsuccessful.

4. Cows fed soybean hay which was harvested after the beans were well formed in the pods, produced butters of somewhat lower vitamin A value than when alfalfa hay was fed.

5. Although soybeans have a suppressing action on the formation of vitamin A in the butterfat, yet it is possible to produce butters of fairly high vitamin A value even when soybeans are used in the rations of the cows provided roughage of high vitamin A potency is fed.

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## PREPARING SAMPLES OF BUTTER FOR ANALYSIS

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Preparing samples of butter for analysis is very slow and tedious work mainly because the butter must be softened and thoroughly mixed. The physical and chemical characteristics of butter are such that it is difficult to hasten the softening without causing part of the sample to melt. When melting occurs there are even greater difficulties of properly mixing the sample to a uniform composition. These difficulties are apparent to the carefully trained technician, and usually the proper precautions are taken to avoid serious errors in the preparation of the sample. There are, however, many buttermakers who analyze each churning and technicians who try to speed up their work, that do not avoid melting of the sample around the edges. In such cases more time and care in mixing the sample is necessary in order to produce a sample of uniform composition. In their haste, these analysts may not use the proper precautions to produce a uniformly mixed sample. Mechanical mixers have been employed by some technicians to ensure uniform mixing, but the proper softening of the butter still seems to be important with at least certain types of mixers. In reviewing the literature, the malted milk type of mixer (1) appears to be the only one which has received much attention although modifications of the horizontal spiral bit type have been used successfully for many years.

A new design of the horizontal spiral bit type of mixer has been made to permit mixing samples of various sizes and is suggested as a possible improvement in the apparatus used for preparing samples of butter for analysis. It consists of an electric motor having a speed of 1725 r. p. m. and a horse-power rating of  $\frac{1}{4}$  horse power. A chuck similar to that on an electric drill is mounted directly on the shaft. A solid center spiral bit which has had the tip or worm and the two cutters removed, is placed in the chuck. When the motor turns, the bit is turned in the opposite direction from that when it is used for boring. This motion throws the butter into the bottom of the sample jar which is slipped over the bit. The bit should be preferably of  $\frac{3}{4}$  inch size and have a solid shaft through the ribs in order to facilitate cleaning. Finally a piece of tin shaped in a half circle is placed on the bench over the bit in order to catch any pieces of butter that may fly off the bit when the sample jar is removed.

Sample jars for use with this type of mixer should be of glass with a tight screw cap, straight sides and flat bottom. The capacity of the sample jar should be approximately 190 per cent of the volume of the sample of butter. The capacity must allow for the volume occupied by the bit. The straight

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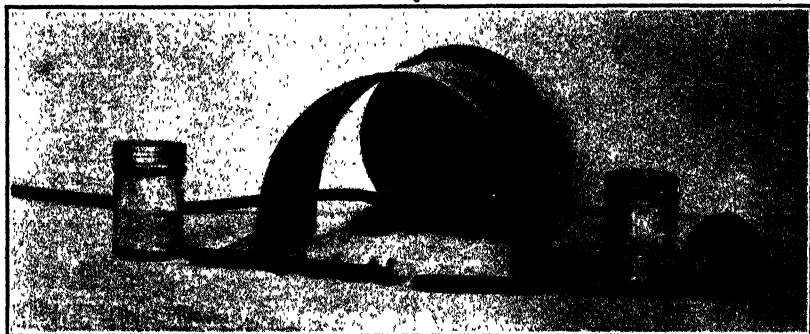


FIG. 1. Horizontal Spiral Bit type of Mixer and Sample Jar. Notice the bit with worm and two cutters removed so that the end is flat.

sides and flat bottom are necessary to permit the bit to reach all corners of the sample jar.

The precautions to observe in using this horizontal spiral bit type of mixer have been established by the following experiments.

#### SIZE OF MOTOR

Samples of butter at 12° C. were mixed with motors of various sizes. It was found that motors rated below 1/10 horse power did not have sufficient power to maintain the speed when the bit was in the butter and the bearings of the shaft were not capable of carrying the weight of the bit.

#### EFFICIENCY OF MIXING AT VARIOUS TEMPERATURES

Each sample was held at its respective temperatures 12 to 24 hours to insure a uniform temperature throughout the sample. The sample was

TABLE 1

*Maximum difference in 8 tests for per cent total solids of samples mixed two minutes at various temperatures*

TIME MIXED IN MINUTES	TEMPERATURE OF SAMPLE		
	12° C.	24° C.	30° C.
2	.22	.24	.24
2	.19	.20	.15
2	.24	.29	.22
2	.31	.28	.43
2	.29	.28	.25
2	.25	.13	.35
2	.19	.26	.29
2	.18	.23	.27
2	.10	.24	.29
2	.16	.22	.36
Average	.21	.24	.29

TABLE 2

*Maximum difference in 8 tests for per cent total solids of samples mixed three minutes at various temperatures*

TIME MIXED IN MINUTES	TEMPERATURE OF SAMPLE		
	12° C.	24° C.	30° C.
3	.08	.07	.19
3	.09	.11	.23
3	.12	.12	.21
3	.11	.10	.18
3	.09	.09	.20
3	.07	.10	.26
3	.09	.08	.19
3	.08	.07	.21
3	.09	.08	.18
Average	.08	.08	.21

mixed for a definite time as shown in Tables 1 and 2. The prepared sample was then analyzed by the Mojonnier Method for total solids, making one determination from each of eight different parts of the sample. The difference between the highest and the lowest results of these eight determinations on the same sample is called the "maximum difference" in the following tables. Obviously, a sample which is uniformly mixed will have a small "maximum difference."

When mixing these samples it was observed that the sample appeared to be uniformly mixed after from one to one-and-a-half minutes of mixing, especially when the temperature was 24° C. or higher. It was also observed that 30° C. usually caused some of the fat to melt around the sides of the sample jar. This partially separated sample, when carefully mixed, had the same appearance as the samples which were mixed at lower temperatures.

The results in Tables 1 and 2 indicate that the sample must be mixed for some time after it appears to be uniformly mixed. Also when the sample has partially separated, mixing for even three minutes will not make it uniform in composition.

It appears that samples mixed at either 12° C. or 24° C. are uniformly mixed, since the "maximum difference" is within the experimental error of the method of analysis.

#### *Effect of Temperature of Mixing Upon Accuracy*

Duplicate samples were prepared from quarter-pound prints of butter by cutting the print into quarters and placing diagonal quarters in a jar for a sample. This gave one-eighth of a pound of butter in each of the duplicate samples. One of the duplicate samples was stored at 12° C. and the other at 24° C. for at least 12 hours in order that the temperature of the entire sample would be uniform.

The samples were mixed for exactly three minutes and then analyzed by

the Mojonnier Method for total solids, making one determination from each of eight different parts of the sample. The average of all eight determinations was assumed to be the correct percentage of total solids of the sample.

TABLE 3

*Average per cent total solids of duplicate samples mixed for three minutes at different temperatures*

12° C. ORIGINAL SAMPLE	24° C. DUPLICATE SAMPLE	DIFFERENCE ORIGINAL MINUS DUPLICATE
84.66	84.61	+ .05
84.63	84.59	+ .04
83.94	84.00	— .06
84.27	84.22	+ .05
84.65	84.71	— .06
84.81	84.78	+ .03
84.69	84.75	— .06
84.83	84.77	+ .06
Ave. 84.56	84.55	+ .01

None of these samples showed any tendency to melt or separate. The "maximum difference" in the results for any one sample did not exceed 0.11.

#### CONCLUSIONS

The following precautions must be observed in using the horizontal spiral bit type of mixer:

1. Use glass sample jar with screw cap, straight sides, flat bottom, and a capacity equal to approximately 190 per cent of the volume of the butter sample.

2. Use a motor of not less than 1/10 horse power and 1725 r. p. m., which can be anchored.

3. Do not permit the sample to partially separate as a result of melting around the edges of the sample jar.

4. Mix the sample for at least three full minutes.

5. Stop the motor after mixing each sample and wipe off the one or two grams of butter which adhere to the bit.

This type of mixer has the following advantages:

1. The sample need not be softened or warmed before mixing provided its temperature is between 12° C. and 25° C. This reduces the opportunity to change the composition of the sample. Samples direct from the churn may be mixed.

2. Less time is required for preparing the sample for analysis.

3. It is simple to assemble, operate, and clean.

4. The butter is thrown toward the bottom of the sample jar thus reducing loss and spattering.

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# THE THIRTIETH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. R. GRAVES  
*Secretary-Treasurer*

The thirtieth annual meeting of the American Dairy Science Association was held at the University Farm, St. Paul, Minnesota, on June 24, 25, 26 and 27, 1935. The program committee, Dr. L. S. Palmer, University of Minnesota, Chairman, arranged programs for the four sections of the association, namely the Manufacturing Section, in which the chief interest is in the dairy products and their manufacture; the Production Section, which is interested in the health, nutrition, breeding and management of dairy cattle; the Extension Section, which is interested in ways and means of presenting educational projects to farmers and the industry; and the Section on Problems and Methods of Instruction, in which the interest is confined to the subject-matter and methods of teaching dairy subjects. The June issue of the JOURNAL OF DAIRY SCIENCE gave the program and in the July issue abstracts of the various papers are given.

The meeting was well attended, as is shown by the following registration by States, prepared by Dr. W. S. Petersen, of the University of Minnesota:

STATE	MEN	WOMEN	CHILDREN	TOTAL
California	3	3	2	8
Colorado	1	—	—	1
Florida	2	2	3	7
Georgia	2	—	—	2
Idaho	1	1	—	2
Illinois	25	7	2	34
Indiana	8	1	—	9
Iowa	15	8	2	25
Kansas	10	4	—	14
Kentucky	5	—	—	5
Louisiana	1	—	—	1
Maryland	3	2	—	5
Massachusetts	4	3	—	7
Michigan	10	1	—	11
Minnesota	102	39	4	145
Missouri	14	5	—	19
Montana	2	1	—	3
Nebraska	8	3	1	12
New Hampshire	4	1	—	5
New Jersey	1	1	—	2
New York	11	5	2	18
North Carolina	3	—	—	3
North Dakota	4	1	—	5
Ohio	15	7	3	25

Oklahoma	5	—	—	5
Pennsylvania	6	2	1	9
Rhode Island	—	—	—	0
South Carolina	6	1	—	7
Tennessee	1	1	—	2
Texas	1	1	—	2
Vermont	1	1	1	3
Virginia	4	—	—	4
Washington	2	1	—	3
West Virginia	1	1	2	4
Wisconsin	25	8	6	39
Wyoming	1	1	3	5
Washington, D. C.	16	7	1	24
Australia	—	1	—	1
Ottawa, Canada	1	—	—	1
Total	325	121	33	479

For a number of years the local committee has arranged programs and trips for the entertainment of the ladies who accompanied the men to the meeting of the American Dairy Science Association. This year one hundred and twenty-one women and thirty-three children registered and were delightfully entertained by the ladies of the local members with the assistance of Minneapolis and St. Paul industries. The program for the ladies follows:

*June 24—Monday*

8:30 P. M.—Informal social get-together for members and their families, Home Economics Building, University Farm.

*June 25—Tuesday*

Hosts for the day—Twin City (St. Paul and Minneapolis) Dairy Industry

9:00 A. M.—12:30 P. M. Sight-seeing automobile trip of the Twin Cities.

12:45 P. M.—Complimentary luncheon, Radisson Inn, Lake Minnetonka.

2:30 P. M.—Return to University Farm Campus.

4:00 P. M.—Members and their families leaves for trip to Land-O-Lakes Creameries, Inc.

6:00 P. M.—Complimentary dinner and entertainment as guests of Land-O-Lakes Creameries, Inc.

*June 26—Wednesday*

Host for the day—General Mills, Inc.

9:00 A. M.—Busses will call for the ladies at University Farm and conduct them on a tour of the University of Minnesota Campus, Minneapolis, and of the Washburn Crosby Flour Mills.

12:00 NOON—Complimentary luncheon at Nicollet Hotel, Minneapolis.

2:30 P. M.—Return by busses to University Farm, St. Paul.

4:00 P. M.—Tea, Fireplace Room, Home Economics Building, University Farm.

Mrs. Walter C. Coffey, Hostess.

5:00 P. M.—Leave for Minneapolis Automobile Club.

7:00 P. M.—Subscription banquet and entertainment at Minneapolis Automobile Club.

*June 27—Thursday*

Shop, golf or other optional activities; or merely rest.

The ladies entertainment committee, that planned and carried out the above program were Mrs. T. W. Gullickson, Chairman, Mrs. Walter Ahlstrom, Mrs. N. N. Allen, Mrs. W. C. Coffey, Mrs. W. B. Combs, Mrs. S. T. Coulter, Mrs. J. B. Fitch, Mrs. E. O. Herreid, Mrs. Harold Macy, Mrs. L. S. Palmer, Mrs. W. E. Petersen, Mrs. B. A. Zakaraisen, Mrs. F. B. Astroth and Mrs. H. R. Searles.

On Tuesday evening the members of the association were the guests of the Land-O-Lakes Creameries, Inc., in an inspection of their large plant and at a dinner at which John Brandt, President of the Land-O-Lakes Creameries, presided. The honor guest was Theophilus L. Haecker, now past 90 years of age, but still alert and active. Professor Haecker was one of the early Professors of Dairy Husbandry in the College of Agriculture of the University of Minnesota. He was a pioneer in the cooperative creamery movement in Minnesota, and was one of the early investigators of the nutritional requirements of farm animals. As a result of his work in the field the Haecker feeding standards were long in use.

On Wednesday noon the association members were the guests of the staff of the University of Minnesota Dairy Department, at a fine luncheon in Haecker Hall.

On Wednesday evening the annual banquet was held at the Minneapolis Automobile Club. The feature of the evening was the presentation of the Association's scroll to Professor Martin Mortensen, Head of the Dairy Industries Department, Iowa State College, as a testimonial of appreciation for his years of outstanding work as a teacher and scientist. Professor E. S. Guthrie, of Cornell University, made the presentation speech. Last year this award was made to Doctor O. F. Hunziker, of Chicago, and the year before to Professor W. F. Fraser, of the University of Illinois. Both of these men were present with their wives and were asked to stand when the scroll was presented to Professor Mortensen. The wording on the scroll follows:

#### MARTIN MORTENSEN

##### *Friend and Councilor*

Professor Martin Mortensen was awarded membership in the Ridder of Dannebrag, the highest honor that the King of Denmark, his native land, can bestow.

Recently he received the honorary degree of Doctor of Laws from Kansas State College of Agriculture and Applied Science, in recognition of his eminent service to the dairy industry.

Two years ago the alumni of his department presented to their alma mater a portrait of their wise and kindly councilor, in honor of his faithful service as Head of the Department of Dairy Industry of the Iowa State College of Agriculture and Mechanic Arts, for a quarter of a century.

In admiration of him and because of his devotion to our Association,

## MARTIN MORTENSEN

is given this tribute at the thirtieth annual meeting at St. Paul, Minnesota in nineteen hundred and thirty-five, by the American Dairy Science Association.

R. R. GRAVES, *Secretary*

C. L. ROADHOUSE, *President*

The opening session was on Monday evening. President L. D. Coffman, University of Minnesota, and Dean W. C. Coffey, University of Minnesota, were introduced by Professor J. B. Fitch, University of Minnesota, and gave welcoming addresses. Dr. C. L. Roadhouse, President of the American Dairy Science Association, gave a brief address and opened a short business meeting at which were given reports of the Editor of the JOURNAL OF DAIRY SCIENCE, Chairman of the Journal Management Committee, and the Secretary-Treasurer. Following this meeting there was an informal social get-together for members, guests and families at the Home Economics Building.

On Tuesday morning at a joint meeting of the American Dairy Science Association, the Corn Belt Section of the American Society of Agronomy, the American Society of Agronomy, the American Society of Horticultural Science, the Great Plains Section of the American Phytopathological Society, the Genetics Society of America, and American Society of Plant Physiologists, a symposium was held on: "Improving the Germ Plasm of Domestic Plants and Animals. Dr. H. K. Hayes, Chairman, Section O, Vice-president, A. A. A. S.; presided. Dean W. C. Coffey, in an introductory address, stressed the fact that perhaps livestock breeders had paid too much attention to points of the show ring rather than to feeding efficiency and added a plea not to "sacrifice superior germ plasm to the inefficient god of the show." Dr. J. L. Lush's address on "accomplishments with animals" followed. Dr. Lush has recently spent a year in Denmark. Many of his illustrations were taken from data derived from Danish cow-testing associations and Danish pig-recording-testing societies. Dr. O. S. Aamodt, University of Wisconsin, gave an illustrated address on the "Analysis and Synthesis in the Development of New Varieties of Plants." One of the interesting features of Dr. Aamodt's address was his description of "weather machines" in which could be reproduced various combinations of weather, and in which varieties of plants could be tested for their ability to withstand high temperatures and low moisture or their hardiness in withstanding low temperatures. Where, under field conditions, it took about six years to reliably establish facts as to the hardiness of a strain it can now be done with the "weather machine" in one year. The symposium was closed by the reading by Dean Coffey of the following letter from Henry A. Wallace, Secretary of Agriculture:

Dear Dean Coffey:

I am afraid I do not have time to prepare a paper on "Future Possibilities in Plant and Animal Production through Genetic Researches." If, however, the following

would be of any service to you, you can incorporate it into your symposium, if you so desire:

Scientific plant and animal breeding is in its infancy. In the animal world especially, most of the significant results thus far have been obtained by private individuals following empirical methods. These methods, in all too many cases, have had more concern with changing the form and appearance than with changing the functions. The accomplishments of the immediate past are sufficiently great so that we can be confident that the potentialities of the future are rich beyond all imagination. But of very necessity, the time factor is such that the progress in any one year or even five-year period will seem distressingly slow.

As we endeavor to segregate superior strains of plant and animal germ plasm and bring about that combination of superior strains which will result in the most efficient functioning organisms, we must remember that not only must there be a carefully planned scientific approach set up over the years but also there must be a profoundly sympathetic attitude toward the organisms with which the work is being done. Scientific analysis is not enough. There must also be love of synthesis on the part of individuals who have a definite affection for the particular species of plant or animal, the innate character of which is being improved. A true plant and animal breeder must also be something more than a Mendelian geneticist. As a matter of fact, the best plant and animal breeders may be only moderately thorough in their understanding of Mendelian genetics. They may be only moderately good in the fields of statistical comparisons, but they must have that sympathetic understanding of life, that highly developed power of observation which characterized the nature students of the nineteenth century and which should now characterize the plant and animal breeders of the twentieth century as they attempt to improve the functions of the organisms in which they are so intensely interested. If the Universities, Experiment Stations, and the Department of Agriculture can furnish facilities to the various individual workers to continue broad-gauged programs over a long period of years, the results fifty years hence, I am convinced, will be truly amazing.

In closing, I would like to express my belief that, in all probability, there will be an ever growing intensity of scientific interest in the functions of living forces as contrasted with the more static inorganic, mechanical forces. As this change in human interest gradually comes to pass, I am sure that life will become definitely richer.

#### PUBLICATION OF ABSTRACTS

O. F. Hunziker reported, as Chairman of the Journal Management Committee, that a plan has been evolved for the Association to publish abstracts of dairy literature. The proposed plan had the unanimous approval of the Board of Directors and after presentation to the convention, was also approved by the membership.

The report of the Secretary-Treasurer was read and approved. The report follows:

#### THE SECRETARY-TREASURER'S REPORT

##### *Membership*

Following is a table showing the membership of the association as of June 15, 1935. The number of new members and associate members that have come in for this year are shown by States together with the number of old members who are in good standing, the total number of members and associate members in good standing by States—and

the number of active members in 1934 who have failed to date to renew their membership for this calendar year.

The totals show that as a result of the campaign for new members this year, 152 new members and 20 associate members have been secured, giving us 640 full members and 21 associate members, or a total of 661 active members to date. This exceeds the total number of active members in 1934 by 39. It may reasonably be expected that the total number of active members for 1935 may go as high as 675.

The unfortunate feature of this showing is that 151 members who were active in 1934 have failed to renew their membership for 1935. These 151 non-renewals constituted close to one-fourth of our 1934 membership.

Our turnover in membership has been high for several years. This year and last we had active and successful campaigns to increase our membership, and the turnover has increased. This failure of so many to renew their membership is probably due in part to economic conditions and in part to the fact that many new members who have joined our association during these membership campaigns have not found in our association or in our Journal what they needed. We are discussing today the possibilities of abstracting the dairy literature for publication in the JOURNAL OF DAIRY SCIENCE. It may be that these abstracts will more nearly meet the needs of many members who are engaged in commercial work than do the technical articles that predominate in the Journal.

We are still far from having the number of members, that the size of our industry warrants, when gauged by the number of men who are interested in or connected with its technical or scientific phases. In January 1930, when I became Secretary, we had about 400 members. The greatest increase in members has come in the past two years, due to the active membership campaigns conducted by Presidents Stoltz and Roadhouse, with the splendid cooperation of members in the various States. If the membership is to be maintained at its present level, or increased, it appears that such membership campaigns will have to become regular yearly affairs.

Some of you who have served on membership committees that have sent in the names of applicants for membership may get the wrong impression from the accompanying table in that new members are credited to the States from which the applicants were recommended. For instance Dr. Ellenberger's Committee in Vermont have sent in a number of applications but the applicants were all residents of States other than Vermont.

*1935 Membership in American Dairy Science Association*

STATE	NEW MEMBERS		OLD MEMBERS		TOTAL		1934 members not re- newed
	Full	Asso- ciate	Full	Asso- ciate	Full	Asso- ciate	
Alabama	1	—	1	—	2	—	—
Arizona	—	—	3	—	3	—	4
Arkansas	—	—	1	—	1	—	1
California	12	1	34	—	46	1	5
Connecticut	1	—	13	—	14	—	4
Washington, D. C.	6	—	17	—	23	—	4
Florida	1	—	4	—	5	—	1
Georgia	—	—	2	—	2	—	1
Idaho	—	—	3	—	3	—	1
Illinois	9	—	39	—	48	—	14
Indiana	1	—	19	—	20	—	4
Iowa	1	—	15	—	16	—	3

Kansas	1	—	8	—	9	—	1
Kentucky	—	—	5	—	5	—	—
Louisiana	—	—	3	—	3	—	1
Maine	—	—	3	—	3	—	—
Maryland	4	—	10	—	14	—	4
Massachusetts	6	—	11	—	17	—	4
Michigan	9	1	17	—	26	1	1
Minnesota	21	—	19	—	40	—	2
Mississippi	—	—	2	—	2	—	1
Missouri	4	—	17	—	21	—	2
Montana	2	1	2	—	4	1	4
Nebraska	3	—	10	—	13	—	2
Nevada	1	—	1	—	2	—	—
New Hampshire	1	—	4	—	5	—	—
New Jersey	4	—	7	—	11	—	4
New Mexico	—	—	2	—	2	—	1
New York	8	—	57	—	65	—	16
North Carolina	2	—	3	—	5	—	1
North Dakota	—	—	3	—	3	—	1
Ohio	7	16	31	—	38	16	15
Oklahoma	2	—	4	—	6	—	1
Oregon	2	—	8	—	10	—	4
Pennsylvania	14	—	27	—	41	—	13
South Carolina	—	—	5	—	5	—	6
South Dakota	1	—	2	—	3	—	1
Tennessee	3	—	2	—	5	—	7
Texas	1	—	3	—	4	—	1
Utah	4	—	3	—	7	—	—
Virginia	3	—	3	—	6	—	2
Vermont	—	—	13	—	13	—	5
Washington	4	—	9	—	13	—	—
West Virginia	1	—	10	—	11	—	3
Wisconsin	5	1	23	1	28	2	—
Wyoming	—	—	1	—	1	—	—
Total	145	20	479	1	624	21	145

*Membership in United States Territories and Other Countries*

	NEW	OLD	TOTAL	DID NOT RENEW
Canada	5	9	14	3
Denmark	—	2	2	—
France	1	—	1	—
Germany	—	1	1	—
Holland	—	1	1	—
Japan	—	1	1	—
Russia	—	1	1	—
Scotland	—	1	1	—
Porto Rico	—	1	1	—
Panama	—	1	1	1

Alaska . . . . .	—	—	—	1
Ireland . . . . .	1	—	1	—
Italy . . . . .	—	—	—	1
	—	—	—	—
Total Foreign	7	18	25	6

	FULL	ASSOCIATE	FULL	ASSOCIATE	NOT RENEWED
United States	145	20	479	1	145
Foreign	7	—	18	—	6
	—	—	—	—	—
	152	20	488	1	151
Total full members			640		
Associate members			21		
			—		
			661		

### Finances

The finances of the association are best shown by the balance sheet as of December 31, 1934, and the Profit and Loss Statement for the Period November 27, 1933, to December 31, 1934. These follow, together with an explanatory letter from Charles E. R. Adams, Public Accountant.

10 Knowles Avenue  
Kensington, Maryland  
February 5, 1935.

Mr. Roy R. Graves  
Secretary and Treasurer  
American Dairy Science Association  
Washington, D. C.

Dear Mr. Graves:

In accordance with your request I have made an examination of the records of the American Dairy Science Association, installed a complete set of double entry books, and submit herewith the following statements and comments:

“EXHIBIT A”—BALANCE SHEET

as at December 31, 1934.

“EXHIBIT B”—STATEMENT OF OPERATIONS

For the period November 27, 1933 to December 31, 1934.

*Comments:*

Cash in Bank as shown by the books was reconciled with bank statements furnished by the McLachlen Banking Corporation. Cash on hand represents one money order, not included in 1934 deposits.

All records furnished me were recorded on double entry books and all accounts were closed at December 31, 1934.

Bonds appear on the balance sheet at par and were recorded according to your information.

Accounts Receivable—Returned Checks were set up from information obtained from your bank statements. As insufficient records were kept of these items it was impossible to trace their final disposition. It is quite probable that the majority of these checks have been made good and the amounts of same included in various deposits. If this

is the case, membership fees or subscriptions are overstated to that degree. However, future adjustments will clear these items in proper order.

I have estimated the inventory taken by Science Press of Journals on hand at December 31, 1934, to be \$525.00. Eighty per cent of this amount has been set up in a reserve to decrease the inventory to a conservative value, future sales of old copies being doubtful.

As there was no inventory of Journals taken at the beginning of the period audited, and the records were incomplete, I was unable to compute the beginning inventory for the purpose of showing the cost of goods sold.

The Profit and Loss Statement shows the amount of purchases as expenses under the various headings. These costs were recorded on a cash basis from the cash book.

Deferred income represents payments made in 1934 on 1935 subscriptions and membership.

In order to facilitate future audits and provide sufficient data for verification of account balances, I recommend the following procedure.

Request your publisher, Science Press, to submit names and addresses of subscribers and advertisers along with their remittances, and record in detail in this report, retail price and discount allowed as well as amount remitted.

Request also a detailed statement of all Journals sold, showing name and address and number of copies of each volume and the same information on advertising contracts. This applies to gratis copies as well.

This information will be of value in the verification of inventory and amounts received.

Respectfully submitted,

CHARLES E. R. ADAMS, *Public Accountant*

AMERICAN DAIRY SCIENCE ASSOCIATION  
WASHINGTON, D. C.

“EXHIBIT A”

BALANCE SHEET  
as at December 31, 1934.

<i>Assets</i>		
Cash in Bank	\$3950.40	
Cash—on hand—undeposited	2.00	\$3952.40
<hr/>		
ACCOUNTS RECEIVABLE—		
Advertising Accounts:		
Creamery Package Mfg. Co.	20.82	
Mitchell Faust	7.00	
Difeo Laboratories	13.00	
Returned Checks	35.00	75.82
<hr/>		
Inventory—Journals (see comments)	525.00	
Less reserve	420.00	105.00
<hr/>		
Investments—Bonds		3000.00
<hr/>		
TOTAL ASSETS		\$7133.22
<hr/>		
<i>Liabilities and Capital</i>		
ACCOUNTS PAYABLE		\$ 955.88
Deferred Income		398.05

**CAPITAL:**

Capital—November 27, 1933 per financial statement of that date	5437.49	
Add: Estimated value of Inventory of Journals at December 31, 1934	105.00	
Net Profit for period, November 27, 1933, to December 31, 1934, transferred from "Exhibit B"	236.80	5779.29

**TOTAL LIABILITIES AND CAPITAL****\$7133.22**

**AMERICAN DAIRY SCIENCE ASSOCIATION  
WASHINGTON, D. C.**

**"EXHIBIT B"****STATEMENT OF PROFIT AND LOSS**

For the Period November 27, 1933 to December 31, 1934.

**INCOME:**

Membership Fees	\$3020.30
Subscriptions	2939.72
Advertising	1375.45
Single Copies	9.50
Back Copies	85.80
Reprints	83.74
Sale of Abstracts	43.53
Sale of Bulletins	50.00
Miscellaneous Income	2.25

**GROSS OPERATING INCOME****\$7610.29****OPERATING EXPENSES:**

Journal	5128.10
Reprints	150.30
Handling of advertising & subscriptions	571.97
Postage & Stationery—Science Press	129.03
Editorial Expense:	
Salary	\$750.00
Telegrams	1.13
Postage & Supplies	89.25
	<hr/>
Presidential Expense	46.50
Secretary and Treasurer	
Salary	300.00
Postage	31.00
	<hr/>
Divisional Allotment	100.00
Accounting & Bookkeeping	100.00
Association Stationery	22.00
Federal Check Tax	1.02
Collection and Exchange	3.61
Miscellaneous	26.20

<b>TOTAL OPERATING EXPENSE</b>		<b>7450.11</b>
<b>NET OPERATING PROFIT</b>		<b>160.18</b>
<b>NON-OPERATING INCOME:</b>		
Bond Interest	112.50	
Bond Premium on U. S. Bonds	35.88	76.62
<b>NET PROFIT TRANSFERRED TO "EXHIBIT A"</b>		<b>236.80</b>

The report of the Auditing Committee also follows:

Mr. R. R. Graves,  
Secretary-Treasurer,  
American Dairy Science Association,  
Bureau of Dairy Industry,  
Washington, D. C.

Dear Mr. Graves:

The auditing committee has examined the books and records of the association for the fiscal year November 27, 1933, to December 31, 1934, and finds them to be correct and in good order. Since the financial statement includes the expenses of 13 months, but the income of only 12 months, the profits for the year 1934 are actually greater than is shown.

The committee recommends that the books be audited annually by a competent public accountant, provided the cost is reasonable (approximately \$25.00).

The committee further recommends that an additional \$1000 be invested in Government Bonds at this time. It feels that purchase of \$2000 worth of bonds at the present time would excessively lower the bank balance which now includes most of the income for the year 1935, but from which the larger part of the year's expenses must still be paid.

Very truly yours,

JOHN I. ORMOND  
(for H. F. Judkins)  
L. A. ROGERS  
B. H. WEBB

At the 1934 annual meeting the Board of Directors authorized the Secretary-Treasurer to change the fiscal year to the calendar year, and to balance the books of the association as of December 31 instead of November 30, as in the past. Consequently the above statement covers a period of 13 months. Some of the operating expenses for the last half of 1933 were paid in December of 1933 and are therefore included in the above statement. The salary of the Editor and of the Secretary-Treasurer as listed on Statement of Profit and Loss covers a year and a half because the salaries for the last half of 1933 were paid in December, 1933. Therefore, the net profit for 1934 is actually nearer \$600.00 than the \$236.80 given in the statement.

For a comparison of the finances of the association, as given on the Balance Sheet as of December 31, 1934, it may be stated that in January, 1930, the capital of the association was \$1,411.17.

The report of the Editor of the JOURNAL OF DAIRY SCIENCE was read and approved. A brief of the Editor's report follows:

The reports of the Editor of the JOURNAL OF DAIRY SCIENCE was received and approved. This report shows that in spite of the monthly publication of the JOURNAL, and its consequent increased expense for the year 1934 as against the bi-monthly publication prior to 1934, the JOURNAL business shows a small profit. This was made possible by the very considerable increase in membership fees resulting from the membership drive that was so successfully conducted during the past year. The report further emphasizes that the increased size of the JOURNAL, the more frequent publication, the increased use of the JOURNAL for Association affairs, and the trend toward an increased proportion of members to subscribers, all indicate an improved JOURNAL and a stronger Association.

The following report of the nominating committee was received and approved:

*For Vice-President:*

J. R. Dice, North Dakota.

R. R. Graves, Washington, D. C.

*For Director for 3-year term:*

J. W. Linn, Kansas.

C. R. Gearhart, Pennsylvania.

Submitted:

H. A. BENDIXEN, Washington

R. B. BECKER, Florida

J. M. SHERMAN, New York

H. B. ELLENBERGER, Vermont

E. L. ANTHONY, Michigan, *Chairman*

BUSINESS CONDUCTED BY THE BOARD OF DIRECTORS

Officers and members of the Board present were: Dr. C. L. Roadhouse, President; H. A. Ruehe, Vice-President; R. R. Graves, Secretary-Treasurer; Professor Martin Mortensen and Dr. O. F. Hunziker, members of the Board. Dr. L. A. Rogers, the third member of the Board, was unable to attend the meeting.

The following motions were adopted:

1. To hold the 1936 annual meeting at Pennsylvania State College, on the dates of June 17, 18, and 19, 1936.

2. To adopt the Registration Fee method of defraying expenses incurred in the holding of the annual meeting. The fee was set at \$1.00 for members and \$2.00 for non-members. Families of members attending the annual meeting will not be required to pay a fee.

3. A vote of thanks was extended to Professor A. A. Borland for the invitation to hold the next Annual Meeting at Pennsylvania State College.

4. The secretary was directed to send the following telegram to Doctor A. C. Dahlberg, Editor of the JOURNAL OF DAIRY SCIENCE. "Directors of American Dairy Science Association express sympathy your illness and regret your inability to attend meeting."

5. The JOURNAL Management Committee was instructed to draw up a new agreement relative to the publishing of abstracts in the JOURNAL, to be submitted to the general meeting for action. (Submitted and approved—see report.)

6. That the JOURNAL Management Committee for the following year be the same as the past year, that is, Dr. O. F. Hunziker, Chairman, Professor A. A. Borland, and the Secretary.

7. The appointment of Professor R. B. Stoltz, of Ohio State University, as Secretary-Treasurer for the next fiscal year—effective January 1, 1936.

8. The reappointment of Dr. A. C. Dahlberg as Editor of the JOURNAL OF DAIRY SCIENCE for the coming year.

At the meeting of the association on June 27 the following business was transacted:

Professor C. Y. Cannon, reporting on the Pasture Committee, requested that the committee be continued and that R. H. Lush, of the University of Louisiana, be reappointed as the association's representative on the joint committee with representatives of the Animal Production Society and the Agronomic Society.

Announcements of the appointment of Professor R. B. Stoltz as Secretary-Treasurer; of the reappointment of Dr. A. C. Dahlberg as Editor of the JOURNAL OF DAIRY SCIENCE; of the acceptance of the invitation to hold the 1936 meeting at Pennsylvania State College on June 17, 18 and 19; and of the adoption of the "Registration Fee" as a means of paying the expenses of holding the annual meeting.

The following report of the Production Section was given by Professor C. Y. Cannon, Chairman.

All papers were presented as listed. Average attendance per session 66. Committee on Nominations were:

C. F. Huffman, Chairman, E. C. Elting and H. P. Davis. The committee presented the following names for election:

*For Chairman*—K. S. Morrow—(elected) New Hampshire.

R. H. Ruffner North Carolina.

*For Secretary*—W. E. Kraus —(elected) Ohio.

S. I. Bechdel Pennsylvania.

Committee on Resolutions were: Fordyce Ely, Chairman, R. B. Becker and T. M. Olson. The committee presented resolutions to the general Resolutions Committee.

Pasture report presented in mimeograph form.

Judging committee reported.

Report moved and accepted.

Judging Committee is as follows: I. W. Rupel, Wisconsin, E. C. Elting, South Carolina, Paul Reaves, Virginia, Elmer Hanson, Iowa, J. F. Kendrick, U. S. D. A., Washington, D. C.

Report of Committee on Breed Relations given by R. T. Harris of Wisconsin—moved, seconded and adopted that breeds take aliquot samples of each milking for composite testing.

Breeds Relation Committee appointed: Roy Harris, Chairman, Wisconsin, S. M. Salisbury, Ohio, Fordyce Ely, Kentucky, E. J. Perry, New Jersey, and C. N. Shepardon, Texas.

The Pasture Committee was reappointed as follows: I. R. Jones, Oregon, G. Bohstedt, Wisconsin, C. B. Bender, New Jersey, R. B. Becker, Florida, and R. H. Lush, Chairman.

The following report of the Extension Section was given by Floyd Johnson, Chairman:

The Extension Section held two half-day sessions and one short session for the explanation of exhibits of teaching materials.

The first session was held on Monday afternoon, June 24th, in order to complete the work of the section and give opportunity for extension members to hear as many papers as possible given by the other sections. The papers given were short reports of committees previously appointed to make studies of the best methods of developing and executing dairy extension projects.

The reports dealt with the following subjects: Exhibits, Quality Improvement in Dairy Products, Sires, Testing, Feeding and 4-H Clubs.

Time was set aside for discussion after each paper. Those attending took full advantage of this opportunity and at times this part of the program became lively.

The officers of the extension section were pleased to see so many members from the other sections attending our meetings.

About 50 attended the two half-day sessions and about 20 attended the short session for the explanation of exhibits.

Last year an exhibit of teaching materials and devices was made by the extension section. This project proved to be of such value that it was repeated this year. The exhibit this year was so much more complete and more interesting than that of last year that the section voted to continue this phase of the program.

There were exhibits from 15 States and the Bureau of Dairying at Washington. Exhibits of publicity materials and dark room was furnished so that film strips could be shown.

The extension section greatly appreciated the attitude of the general program committee in assisting with the making of the extension program.

This section also appreciated the excellent arrangements provided for exhibits and meetings.

The extension section passed a resolution commending the association for arranging for a section on problems and methods of instruction. The extension group feels that this idea should be further developed to include all phases of dairy teaching. We believe

that the teaching of agriculture and dairying in particular offers a real opportunity to provide education in its broadest sense.

The events of the last few years show that we need to educate leaders in dairying who are not only well trained in that field, but who are also broad-gauged members of society.

In 1934 there were 43 extension members of the American Dairy Science Association—in 1935 there were 56. Only 3 members failed to renew their membership this year.

There are 110 dairy extension men in the United States according to extension lists furnished by the Bureau of Dairying.

Officers elected for 1936 were:

E. J. PERRY, New Jersey, *Chairman*  
C. L. BLACKMAN, Ohio, *Vice-Chairman*  
EARL SHULTZ, Iowa, *Secretary*

The following report of the Manufacturing Section was given by Professor G. N. Trout, Secretary:

#### REPORT OF THE DAIRY MANUFACTURERS' SECTION

Due to the able work of Chairman Mack and to the cooperation of the several members presenting papers, the full program of the Dairy Manufacturing Section has been carried out according to schedule. With the exception of two papers withdrawn, the program was carried out as printed.

At the business meeting, Dr. E. S. Guthrie, Chairman of the general committee on "Chemical Methods for the Analysis of Milk and Dairy Products," reported that progress was being made by each of the seven subcommittees on Milk, Butter, Cheese, Ice Cream, Condensed and Evaporated Milk, Dry Milk, and Skim Milk, Buttermilk, and Whey. He reported that four of the committees had been very active and would soon have their findings in published form.

Dr. Harold Macy, reporting for the general committee on "Bacteriological Methods for the Analysis of Milk and Dairy Products," stated that the committees on Milk, Butter, Cheese, Ice Cream, Condensed and Evaporated Milk, and Dry Milk had reported progress.

Prof. H. W. Gregory, chairman of the committee on "Judging Dairy Products," summarized the work of the committee and pointed out the interest being shown in the judging of dairy products as evidenced by the increase in the number of judging teams participating in the national contest.

Dr. H. E. Van Norman discussed briefly the educational work of the Chicago Mercantile Exchange. Chairman Mack appointed a committee, composed of Prof. M. Mortensen, chairman, Dr. O. F. Hunziker, and Prof. P. S. Lucas, to work with Dr. Van Norman to investigate the possibilities of a plan for recognizing the buttermaking efficiency of butter makers in the several states now sponsoring state scoring contests.

Dr. L. M. Thurston, West Virginia, and Dr. W. V. Price, Wisconsin, were elected chairman and secretary respectively for the Manufacturers Section for the coming year.

G. M. TROUT  
*Secretary, Manufacturing Section*

A report of the hotel arrangements for the association in St. Louis at time of the National Dairy Show next October was given by Professor Ragsdale.

Professor Gregory gave a report on the Products Judging Contest.

There was some discussion concerning the possibilities of a banquet at the Dairy Show next fall at which both the dairy cattle contest winners and the products contest winners would be honored.

Mr. Van Norman offered the following motion—which was adopted—that Professors Gregory, Ruehe, and Ragsdale constitute a committee to work out arrangements for a joint banquet that would be in accordance with the sentiment expressed at this meeting.

Professor Fitch, Chairman of a Committee on Milk Standards, stated that the committee was not ready to report and requested that the committee be continued.

The following Report of the Resolutions Committee was read by Professor Borland and approved by the Association :

Whereas the American Dairy Science Association assembled at the University of Minnesota, College of Agriculture, University Farm, has enjoyed a most satisfactory convention professionally and socially, therefore be it resolved

(1) That the appreciation of the membership be tendered to the personnel of the University of Minnesota and College of Agriculture and to the following organizations which have contributed to the success of the 30th annual meeting:

Dairy Record.

Dairy Supply Houses.

General Mills, Incorporated.

Land O'Lakes Creameries, Incorporated.

Minneapolis Milk Distributors.

Minnesota Creamery Operators' and Managers' Association.

Northwest Ice Cream Association.

St. Paul Milk Distributors.

Twin City Milk Producers' Association.

Twin City Unit of the National Dairy Council.

Twin City Press.

(2) That in view of the fact that more attention is now being given to the fundamental breeding of all classes of live stock including dairy cattle, we recommend that more effort be directed toward the keeping of continuous production records of dairy cattle through the herd test and Dairy Herd Improvement Associations. We commend with favor any effort that can be made toward increasing financial support for this work from available State and Federal funds.

(3) That we earnestly urge that definite effort be made to improve the educational value of the dairy cattle judging contests as sponsored by this association and held in connection with the National Dairy Show. These contests in the past added much enthusiasm and interest among our students. We now have available knowledge which makes possible the development of a contest which will take into consideration the economic production ability and genetic make-up of our dairy cattle in addition to the appraisal of the value of dairy cattle on conformation. The form of contest approved by the American Dairy Science Association will influence the procedure followed in similar contests held under the direction of the 4-H club and vocational high school organizations.

(4) That we commend the Food and Drug Administration of the United States Department of Agriculture for its effort to improve the quality of dairy products and also commend the efforts of all organizations and individuals of the dairy industry that are making constructive efforts to aid in the improvement of the quality of all dairy products,

and further commend the payment to the producer of a differential for the delivery of quality milk and cream.

O. E. REED

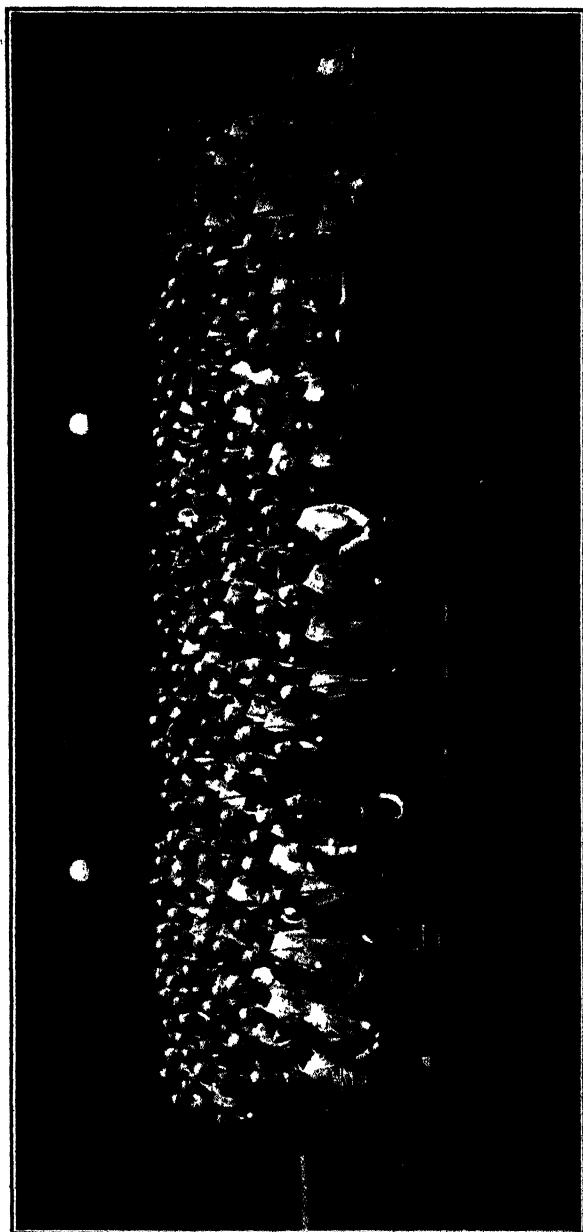
H. E. VAN NORMAN

W. J. FRASER

J. A. NELSON

A. A. BORLAND, *Chairman*

Adjournment.



# AMERICAN DAIRY SCIENCE ASSOCIATION MEMBERSHIP LIST AS OF JULY 15, 1935

## A

ABBOTT, FRED H., Dairy Industry Division, College of Agri., Davis, Calif.  
 ACKERMAN, RICHARD A., Reymann Memorial Farms, Wardensville, W. Va.  
 ADAMS, HAROLD S., Nashoba Assoc. Board of Health, Town Hall, Ayer, Mass.  
 ADDINGTON, LAWRENCE H., New Mexico A. & M. College, State College, New Mexico.  
 AHLSTRAND, ERIC R., State Dairy & Food Inspector, Zumbrota, Minn.  
 AHLSTROM, WALTER, 3800 Lyncale Ave. N., Minneapolis, Minn.  
 AIHRENS, ALFRED H., 1179 Forest Ave., Bronx, New York, N. Y.  
 ALEXANDER, C. B., The Akron Pure Milk Co., 273 Bowery St., Akron, Ohio.  
 ALGER, HARRY B., 13505 Griggs, Detroit, Mich.  
 ALLEN, ARTHUR E., 100 Shell St., Progress, Pa.  
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## INVESTIGATION OF RESAZURIN AS AN INDICATOR OF THE SANITARY CONDITION OF MILK

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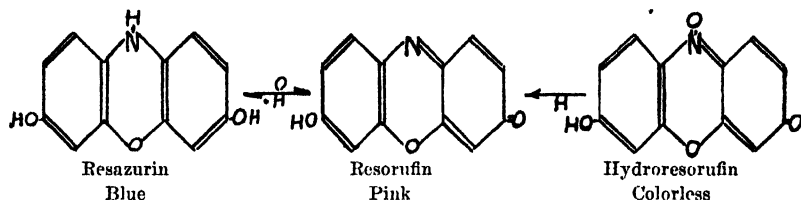
The use of chemical indicators for determining the sanitary condition of milk is finding considerable application. It is the purpose of this paper to describe our studies of the indicator resazurin. On the basis of the results of this investigation extending over two years, it is felt that more information as to the sanitary condition of milk can be obtained by the use of resazurin than from any other chemical indicator now in use.

While methylene blue fulfills quite well all the requirements of a reduction test as specified by Thornton and Hastings (1), it still falls far short of giving a true picture of the sanitary condition of many milk samples. Its time of reduction is dependent, in part, upon the rapidity of growth of those organisms that develop under a definite temperature of incubation and not upon the actual number of bacteria initially present,—that is, the original contamination. Also, it fails to classify properly those milks containing organisms that are slow reducers, since a population well into the millions must be developed before the Eh of the milk is sufficiently low to effect complete decolorization. Differences in reducing intensities of different organisms have been observed by Clark and his associates (2) and by Frazier and Whittier (3). Another objection is that the test takes too long. Thornton and Hastings (1) state that any oxidation-reduction indicator more electro-positive than methylene blue will give misleading results. Clark (2), on the other hand, has expressed the belief that a more electro-positive indicator might be advantageous. Our results, with the slightly more electro-positive indicator resazurin, bear out Clark's opinion. This is especially true if the sanitary condition of milk is to include the pathological and physiological abnormalities as well as the bacterial contamination.

Resazurin as an indicator of the sanitary condition of milk has received some attention abroad, but there has been no reference in the literature to its use for this purpose in this country. Comparisons of reduction time of resazurin and bacterial numbers were made by Olga Waldbauer (4).

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This author, on the basis of her results, was able to classify milks into four groups. Mundiger and Wolf (5) compared the reduction time of resazurin with plate and Breed counts. They conclude that claim cannot be laid to an accurate bacterial count by the resazurin test, but that the test is a good means of approximating the quality of milk, is applicable to control work, and is especially useful for rapidly detecting poor milk of high bacterial numbers. These authors state that resazurin in milk reduces to resorufin, which, in turn, reduces to the colorless modification hydroresorufin.<sup>1</sup> These steps are shown below.



The reduction of resazurin in milk is not reversible, while the change of resorufin to hydroresorufin is reversible. Resazurin in milk at a pH of 6.5 is blue and at a pH of 5.3 is red, while resorufin is pink at a pH of 6.5 and yellow at a pH of 4.8. A mixture of resazurin and milk that is becoming progressively more negative in Eh will change from blue to pink through various shades of purple and lavender. The dye has a high tinctorial power and the color changes are easily judged.

#### EXPERIMENTAL

It was found that an aqueous solution of resazurin containing .05 per cent of the dye did not undergo any visible decomposition when confined in the dark for six months. Consequently, a solution of this concentration was used for stock solution, and when 0.1 ml. was added to 10 ml. of milk the color produced was sufficient for good tinctorial effect. The germicidal effect of this concentration, *i.e.*, one part of dye to 200,000 parts of skim milk, was then determined. The results are given in Table 1. It is observed that resazurin does produce a significant toxic effect in milk, but methylene blue in concentrations of one to 571,000 suppressed growth practically to the same extent. However, this toxicity does not seriously operate against the use of resazurin as a reduction test for sanitary condition. In fact, the stock solution is not germicidal for all organisms, hence considerable care must be exercised to keep it sterile.

<sup>1</sup> We have been unable to obtain the same shade of pink as developed when resazurin-milk mixtures are incubated by adding the chemical resorufin to milk in like proportion. This seems to throw some doubt upon whether the difference is due to the resorufin used or whether resazurin actually changes to resorufin during the course of reduction. These authors state that their dye contained pure resazurin together with a small percentage of resorufin.

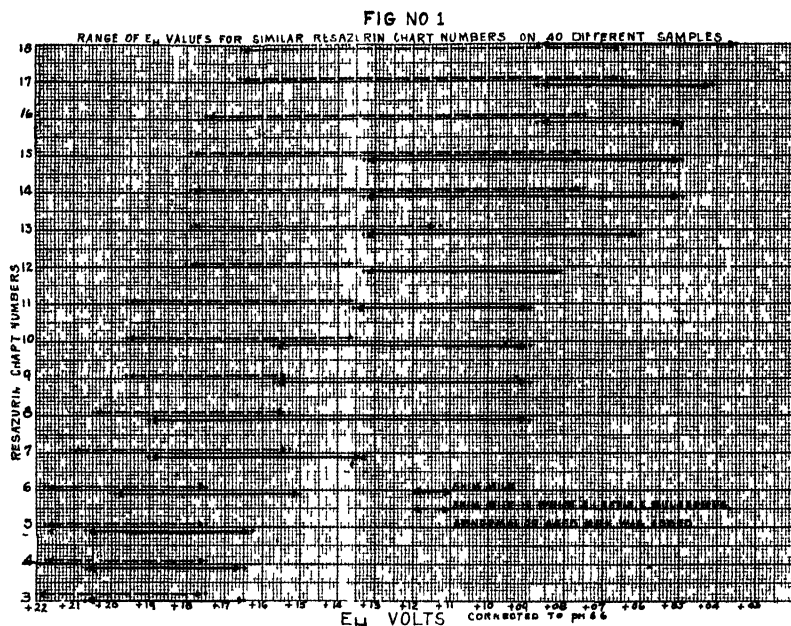
TABLE 1  
*Germicidal effect of resazurin and methylene blue in skimmed milk*

SERIES NO.	HOURS INC. AT 37° C.	MICROSCOPIC COUNT IN MILLIONS				% REDUCTION IN MULTIPLICATION BY ADDIT. OF IND.		
		CONTROL	Resaz-Skim. 1 : 200,000	Resaz-Skim. 1 : 400,000	MB-Skim. 1 : 571,000	Resaz-Skim. 1 : 200,000	Resaz-Skim. 1 : 400,000	MB-Skim. 1 : 571,000
1	0	2.6	2.8		2.8			
"	3	110.0	78.0		76.0	30.0		31.9
2	0	2.9	3.3	2.8	3.2			
"	3	130.0	100.0	110.0	110.0	22.9	15.6	15.9
3	0	2.5	2.4	2.4	2.2			
"	3	63.0	59.0	60.0	52.0	3.6	2.7	9.0
4	0	1.6	1.8	1.6	1.7			
"	3	84.0	56.0	70.0	58.0	33.5	17.0	31.7
"	5	800.0	660.0	640.0	680.0	17.6	20.0	15.0
5	0	3.6	3.0	3.6	3.5			
"	3	78.0	59.0	80.0	64.0	24.7	0	18.7
"	5	700.0	500.0	500.0	450.0	28.6	28.7	35.9
6	0	.1	.1					
"	3½	12.5	7.6			39.5		
"	4½	13.6	9.4			31.1		
"	5	27.0	17.0			37.2		
7	0	.096	.08					
"	3½	1.0	1.6			40.5*		
"	5½	40.0	28.0			30.0		

\* Increase.

To identify the various colors developed in resazurin-milk mixtures during incubation, comparisons were made with a color chart. The chart contained 10 different color standards, each color being given an odd number in increasing sequence to its diminishing proportion of blue, so that the last standard was numbered 19 and was entirely pink. Standard No. 1 corresponded to the color produced by adding resazurin to fresh skim milk in concentration of one of dye to 200,000 of milk. While the number of standards used in the chart was chosen arbitrarily, and possibly is greater than would be considered necessary, especially by one running a large number of tests daily, it was found that there was sufficient variation in color between any two consecutive standards to be easily distinguished. Likewise, the more standards, the more accurate would be the determination of the amount of reduction. The standards were prepared by filling 20 ml. test tubes with mixtures of various proportions of undyed china clay and china clay dyed with brom cresol green, crystal violet, and alkaline phenolphthalein.

Comparisons were made between Eh values measured potentiometrically during the potential drift and the developed colors on 40 different samples of resazurin-skim milk mixtures. There was quite a spread in Eh values corresponding to a single resazurin number, so that it was impossible to interpret intensities on the basis of the developed color. The maximum and minimum Eh values obtained for each resazurin number on this series of samples are presented in Figure No. 1. Eh measurements were not



obtained for all of these extremes, this procedure being impracticable; consequently, they are not entirely valid for each color number. However, the variations between the true and plotted values should not vary by over .005 volt. The zone of reduction of resazurin to resorufin as determined visually by the disappearance of the blue modification in 26 samples of skim milk in which no abnormal milk or special cultures had been added was never more positive than +.10 volt, or more negative than 0 volt. The zone of decolorization of resorufin of these samples fell within the Eh range of +.05 and -.05 volt. In market milk the zone of reduction of these two systems is approximately .1 to .05 volt more positive. In all the physiologically abnormal and pathological milks<sup>2</sup> tested the zones of reduction were shifted to even more positive values.

<sup>2</sup> Throughout this paper the term "physiologically abnormal milks" refers to colostrum and milk from cows drying up, and "pathological milks" refers to milk from cows with diseased udders.

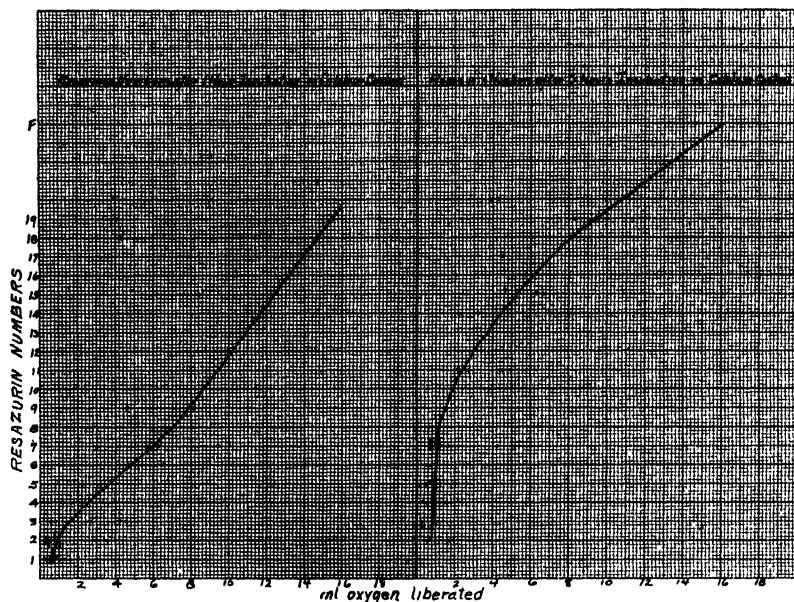
If an oxidation-reduction indicator were used to measure only bacterial contamination, then even methylene blue, which in some milks decolorizes at Eh levels as positive as the upper limits of milk systems (1), *i.e.*, from +.2 to +.3 volt, would give misleading results, and obviously indicators more electro-positive would be even less dependable. However, leucocytes or substances associated with leucocytes reduce methylene blue and if they do not produce a corresponding lowering in oxygen level, then their presence might account, to some extent at least, for the shifting of the zone of methylene blue or resazurin reduction to more positive potentials. Consequently, if this shift is produced by substances present only in physiologically abnormal and pathological milks, then it seems that an indicator such as resazurin, which is reduced by these abnormal milks, and, being slightly more electro-positive than methylene blue but more electro-negative than normal, fresh milk, would respond more rapidly to the reducing influences of abnormal milks and thus, instead of yielding misleading results, be of added value as an indicator of quality over that of methylene blue. Also, with a dye such as resazurin which reduces irreversibly to another colored compound, any slight reduction would be manifest in an alteration of color and thus yield a measurable record of any abnormality or of any slight potential drift.

#### COMPARISON OF RESAZURIN REDUCTION AND VARIOUS TESTS USED TO DETECT ABNORMAL CONDITIONS OF MILK

In order to learn to what extent resazurin is reduced by physiologically abnormal and pathological milks, comparisons were made between the rate of reduction of resazurin and tests used to detect abnormal milks. Middle milk was used and was obtained from individual quarters. So as to eliminate so far as possible the reducing effect of fortuitous bacterial contamination, samples were drawn directly into sterile bottles. The milk was morning's milk and only a few hours had elapsed from the time of milking until the tests were made. The tests included analyses for catalase and chlorides, cell counts, microscopic counts before and after five hours incubation at 37° C., and standard agar plate counts, together with methylene blue and resazurin reduction tests. The course of reduction of the resazurin samples was determined by comparison with the color chart every hour for five consecutive hours. It was observed that in every sample of high resazurin number, *i.e.*, 11 or over within the first two hours, there was also a high cell count of one million or more, regardless of the bacterial analysis.

The relationship between resazurin reduction and catalase content of these 49 samples is shown in Figure no. 2. In the samples of low catalase and small bacterial population only a slight resazurin reduction resulted even after three hours of incubation. However, a high catalase content was invariably associated with considerable reduction of this indicator.

FIG. NO. 2



On the other hand, where the reduction of resazurin was high without a correspondingly high catalase content, the divergence resulted from the fact that these milks were of high bacterial numbers. Methylene blue decolorized in less than eight hours in only two of the 49 samples. One sample containing 16 million cells did not fade methylene blue before eight hours, but the resazurin number after only one hour of incubation was 15, indicating considerable reduction.

While it is still a mooted question whether high leucocyte content in milk is deleterious, especially if the leucocytes do not come from suppurative discharges, the mere existence of large numbers is certainly indicative of the fact that the milk is abnormal. And since it has been shown by Rosell (6) that mastitis milk is quite prevalent, a test that responds to this type of milk as well as to bacterial contamination is of additional value as a test of quality over that with methylene blue.

#### COMPARISON BETWEEN METHYLENE BLUE AND RESAZURIN TESTS

Comparison between methylene blue and resazurin tests were made on 220 market milk samples. The methylene blue solution was prepared by dissolving one methylene blue tablet in 200 ml. of sterile water. One ml. of this solution was added to 10 ml. of milk. The resazurin numbers were obtained by comparing the developed color with the color chart after the

resazurin-milk mixtures containing one of dye to 200,000 of milk were incubated for one hour at 37° C. From the results obtained the milks were graded into four classes according to the following scheme:

*Methylene Blue Reduction Time	Class	Resazurin Number After One Hour Incubation
More than 5½ hours	1	1 to 6
2 to 5½ hours	2	7 to 18
20 mins. to 2 hours	3	19 to nearly faded
Less than 20 minutes	4	Completely faded

Comparison of 410 additional market milk samples were made. In this latter series the methylene blue concentration in milk was in the ratio of one to 200,000. This methylene blue-milk mixture contained 2.85 times more methylene blue than that used in the standard method where methylene blue tablets were used. In this latter series Class 1 with the resazurin test included all colors from 1 to 7, Class 2 numbers 8 to 18, and the remaining two classes similar to the former classification. The results of these two series of comparisons are given in Table 2.

TABLE 2  
*Comparison between methylene blue and resazurin tests*

CLASSIFI- CATION BY RESAZURIN TEST	CLASSIFICATION BY METHYLENE BLUE TEST CLASS								Totals	
	No.	%	No.	%	No.	%	No.	%	No.	%
Class 1 <sup>1</sup>	66	30.0	27	12.3					93	42.3
2	25	11.4	53	24.1	3	1.3			81	36.8
3			13	5.9	21	9.5			34	15.4
4					7	3.2	5	2.3	12	5.5
	91	41.4	93	42.3	31	14.0	5	2.3	220	100.0
Class 1 <sup>2</sup>	263	64.1	15	3.7					278	67.8
2	59	14.4	57	13.9					116	28.3
3			9	2.2	5	1.2			14	3.4
4							2	.5	2	.5
	322	78.5	81	19.8	5	1.2	2	.5	410	100.0

<sup>1</sup> The ratio of methylene blue to milk was 1: 571,000.

<sup>2</sup> The ratio of methylene blue to milk was 1: 100,000.

In the first series of comparisons there were 27 samples that graded Class 2 with the methylene blue test and Class 1 with the resazurin test. From the low resazurin numbers after one hour of incubation it can be reasoned that cells were comparatively few in these milks and bacteria had multiplied very little during the first hour of incubation, but had

\* Classification given in Standard Methods of Milk Analysis, p. 41, published by the American Public Health Association, sixth edition, 1934.

multiplied sufficiently thereafter to decolorize the methylene blue within two to five and one-half hours. We feel that the fact that the resazurin test classifies these milks as it does, rather than disqualifying the test is an advantage, since the number of organisms originally present in the milk, that is, its original contamination, is not always correlated with the methylene blue test, especially in good quality milks. Also, the conditions to which the milk is subjected in conducting the methylene blue test differ considerably from those normally used by the consumer, especially in regard to holding temperature.

By making two observations in the resazurin test, the first after one hour of incubation and the second after two hours, a more nearly complete picture of the quality of milk could be obtained. However, this procedure lengthens the time of the test, which is not always desirable. One hour of incubation is generally sufficient to detect most physiologically abnormal and pathological milks, as well as milks containing actively growing organisms. Of course, those milks that contain sluggish organisms that do not start multiplication until after the milk has been incubated for over one hour would escape detection. The test would have to be altered to include more observations at longer incubation periods to enable a proper grading of these types of milk. By applying the resazurin test so that only one observation is taken, considerable time is saved over that required by the methylene blue test.

#### THE USE OF RESAZURIN TO DETECT SANITARY CONDITION OF HERD MILK SAMPLES

In Table 3 is presented a series of tests on herd milk samples, including microscopic counts, cell counts, methylene blue reduction tests (concentration of 1 to 200,000), and resazurin reduction numbers read every hour for four hours.

While, as previously stated, it is impossible to ascertain the oxidation-reduction potential of a milk-resazurin mixture by its developed color, the rate of change of color should be some indication of its bacterial development. However, due to differences in reducing intensity of organisms and the sensitivity of resazurin to certain unknown factors not necessarily associated with bacterial flora, a true correlation between the rate of resazurin reduction and the rate of bacterial multiplication is not to be expected. Nevertheless, considerable information can be obtained as to the flora of the milk by observing the rate of reduction as manifested in the change of color from blue to pink during incubation.

To illustrate, consider sample No. 734. The resazurin number after one hour of incubation was only 3, indicating very little bacterial multiplication, and the number only increased from 3 to 7 within the next two hours, which still indicated very little bacterial multiplication, but between

TABLE 3  
Comparison of resazurin reduction tests with other quality tests on market milk

PATRONS NO.	MICROSCOPIC COUNT IN THOUSANDS	CELL COUNT IN THOUSANDS	METH. BLUE REDUCT. TEST 1: 200,000 HOURS	RESAZURIN TEST—CHART NUMBERS HOURS INCUBATED—37° C.					
				1	2	3	4	6	7
725	250,000	450	7/12	F					
937	40,000	1,300	17/12	20	F				
941	9,000	300	13/4	F					
654	7,000	450	25/6	15	20	F			
876	6,500	1,600	3	17	F				
839	4,500	1,800	5	7	17	22	F		
924	3,600	380	37/12	20	4/5 F	F			
756	3,600	350	62/3	13	17	19	4/5 F		F
804	3,500	1,400	32/3	15	19	22	F		
821	2,500	730	31/3	5	17	4/5 F	F		
771	1,900	540	71/6	13	19/11	F/11	4/5 F		
624	1,500	800	31/2	11	20	22/F	F		
822	1,500	270	4	5	20	4/5 F	F		
788	760	930	121/2	7	9	9	15		F
788	700	1,400	10	6	8	13	15		F 9 hrs.
734	640	540	75/12	3	6	7	15		1/2 F
741	600	300	51/6	5	15	20	F		
718	590	1,000	81/4	2	4	7	11		16
744	590	400	10	3	5	6	9		
915	570	400	111/4	3	3	3	4		9
873	560	2,000	71/2	12	17	19	19	F	
873	420	820	81/2	5	11	15	16		17
842	300	760	91/2	5	5	9	13		F 9 hrs.
826	250	1,000	111/2	4	11	15	17		18
702	220	1,200	10	3	5	5	7		
814	200	80	115/16	2	3	7	7		11
627	190	860	51/3	15	19	F/22	F		
914	190	570	105/12	3	3	4	11		
687	170	2,400	92/3	12	17	19	20		4/5 F
771	160	360	9	5	8	12	13		F 9 hrs.
972	160	580	71/4	4	11	19/15	20/17		22
672	140	1,300	55/6	3	7	13	20		F
677	140	320	10	2	3	5	11		F 9 hrs.
602	140	800	8	4	7	12		17	
756	130	280	8	2	4	7		12	
750	110	3,200	51/12	15	18	20	22		F
934	110	2,500	71/2	6	11	15	18		18
755	96	400	8	4	5	7	15		23
877	96	170	71/2	3	3	6	7		18
842	80	550	7	3	5	7	16		22
786	64	2,400	8	10	15	17	19		F
810	64	1,200	115/6	7	15	17	18		21
662	64	680	8	4	7	12		19	
799	48	1,200	10	5	9	13	15		
701	32	1,400	101/4	12	16	18	19		22
876	32	1,200	10	9	15	17	18		
667*	32	620	81/6	17	20	21	21		22
677	32	110	115/12	3	4	5	11		17
633	16	1,500	4	5	15	16	19		22
618	16	480	8	2	6	12		19	
786	16	300	8	2	5	7		15	
687	16	3,000	10	9	15	17	18		
985	16	2,200	93/4	7	15	18	19		21
877	16	1,600	10	5	12	14	15		1/2 F
926	16	1,300	8	4	11	15		15	
754	16	1,300	10	5	8	9	13		
644	16	1,200	91/2	5	12	14	17		

TABLE 3—(Continued)

*Comparison of resazurin reduction tests with other quality tests on market milk*

PATRONS NO.	MICROSCOPIC COUNT IN THOUSANDS	CELL COUNT IN THOUSANDS	METH. BLUE REDUCT. TEST 1:200,000 HOURS	RESAZURIN TEST—CHART NUMBERS HOURS INCUBATED—37° C.					
				1	2	3	4	6	7
926	16	1,300	11 1/4	6	13	15	18		18
644	16	1,000	9	3	10	15	17		1/4 F
862	16	1,000	8	3	6	11		15	
720	16	930	7 1/2	5	15	17	20		F
702	16	810	9 1/2	3	5	5	12		20
936	16	800	8	2	10	13		21	F
672	16	720	10	2	5	5	5		
764*	16	700	8	7	13	15	17		23
811	16	600	10 2/3	3	5	5	13		15
801	16	520	8	2	4	5		10	
909	16	510	10 1/4	5	5	13	15		17
650	16	380	9	5	13	19/12	19		F
801	16	380		3	4	5	11		15
899	16	280	8	2	6	10		19	
814	16	280	10	2	3	5	6		F 10 hrs.
741	16	240	7 1/2	2	3	5	8		F 6 1/2 hrs.
608*	16	240	8 1/2	9	15	17	18		22
947	16	220	6/11/12	2	3	3	4		11
656	16	200	12 2/3	3	4	7	13		17
792	16	120	8	2	4	6		17	
777	16	112	6 5/6	3	6	15			F

Resazurin numbers greater than 19 indicate partial fading of the pink modification, the larger the number the more faded.

\* No. 764—Chloride .137%  
 608— " .138%  
 667— " .130%

F—faded

the third and fourth hours the resazurin number changed from 7 to 15. Within this fourth hour the bacterial population must have increased considerably to account for this rapid change in resazurin reduction, but the flora was not of the fast reducing type, since over seven hours elapsed before the dye finally began to fade. The methylene blue sample did not decolorize before 7 5/12 hours. The microscopic count before incubation was 640,000, and in view of this rather high count and low resazurin number after one hour of incubation, many of the organisms must have been in the resting stage or dead.

In several of the samples the resazurin numbers are considerably greater than would be expected from the microscopic count. In most of these samples there are rather high cell counts. In general, wherever there has been a rapid reduction of resazurin within the hour of incubation in samples of milk of low bacterial numbers, either some abnormal feature of physiological or pathological origin has been found or a rapid multiplication has occurred. On the other hand, some abnormal milks have not always produced a marked reduction of resazurin within the first hour of incubation.

## RECOMMENDED PROCEDURE FOR CONDUCTING THE RESAZURIN TEST

The following procedure for the resazurin test is suggested. Prepare a .05 per cent aqueous solution of resazurin by dissolving the dye in hot, distilled, sterile water, in a volumetric flask of suitable capacity. Cover the flask and cool, then fill to mark with sterile, distilled water. To keep this stock solution sterile pour approximately the amount desired into a sterile bottle, then flame the neck of the flask, cover with a sterile stopper and place in the dark until the following day or when again needed. Use the solution transferred to the sterile bottle for the day's run. The amount of solution required for each milk sample, which is 0.1 ml. per 10 ml. milk, can be either measured out by using a pipette calibrated in 0.1 ml. divisions, or a pipette with a tip tapered to deliver the required amount in exactly whole drops, *i.e.*, not fractions of a drop.

To eliminate the necessity of tipping the test tubes containing the milk and resazurin solution to effect uniform distribution, the dye solution should be added to the test tube first and the milk then poured in quite rapidly. However, where it is not practicable to follow this technique, the tubes must be turned upside down to obtain uniform mixing. Since the results are only comparative, either method is satisfactory as long as the technique used is consistent.

Incubate the mixtures at 37° C., in a bath kept in a dark portion of the room, or, if this is impossible, cover the bath with a black cloth or any suitable cover which will shut out the light. After one hour of incubation, compare the developed colors with the color chart and from readings obtained group the samples into the following four classes:

Class 1—Good milks, colors between 1 and 6.

2—Fair milks, colors between 7 and 18.

3—Poor milks, colors pink but not faded.

4—Very poor milks, decolorized.

By returning the samples to the bath and recording hourly changes until the dye has decolorized, a more precise analysis of the sanitary conditions of the milks may be obtained. A microscopic examination will aid in arriving at a correct interpretation of the results. See discussion of sample No. 734, as an example.

The following outline enumerates the main conditions that may be encountered:

1. If the reduction is rapid, commencing at or soon after incubation,

Significance:

- (a) Milks highly contaminated with flora in actively growing state.
- (b) Organisms strongly reducing.
- (c) Pathological milks.
- (d) Physiologically abnormal milks.

2. If the reduction is slow over four to six hours of incubation,

Significance:

- (a) Low bacterial count.
- (b) High bacterial count with either dead or inactive organisms.
- (c) Low cell count.
- (d) Very little multiplication of organisms.
- (e) Much development of bacteria with weakly reducing characteristics.

3. If the reduction is first slow, then followed by a rapid reduction,

Significance:

- (a) Physiologically normal milk.
- (b) Not pathological milk.
- (c) Low bacterial count.
- (d) High bacterial count with organisms in lag phase.
- (e) During period of rapid reduction bacteria are on the threshold of or in the logarithmic growth phase and the speed of reduction is a measure of their reducing power.

4. If the reduction is at first rapid and then followed by a slow reduction,

Significance:

- (a) Physiologically abnormal milks.
- (b) Pathological milks.
- (c) High bacteria count, but poor reducers if well advanced in their logarithmic growth phase.
- (d) Multiplication stopped soon after incubation.
- (e) Very often old milk in which some multiplication had occurred.

Satisfactory colors can be obtained for the color chart by using the dyes described in this paper. However, there are, no doubt, many other dyes, as well as other vehicles, that could be used to produce a chart with colors similar to those developed when resazurin undergoes reduction in milk. Sterilized milk has not been found to be satisfactory as a vehicle, since the yellow color produced during sterilization alters the shades to the extent that they will not match the colors developed during incubation of the mixtures.

The yellow pigment in milk lessens the blue intensity of the resazurin-milk mixtures, the amount depending upon the concentration of pigment. As the yellow pigment, carotene, is fat soluble, it will be carried to the top with the fat, so after the sample has been held for one hour and the fat collected at the top, the milk-resazurin colors approximate more nearly the colors obtained when adding resazurin directly to skim milk. Consequently, the color standards should be made to compare with skim milk-resazurin mixtures rather than with whole milk-resazurin mixtures.

#### SUMMARY

From the results of this investigation the following conclusions are drawn:

1. Only one hour is required to complete the resazurin test as prescribed in the text, while the methylene blue test requires over five hours.

2. Milk can be classified into four groups as regards sanitary condition by means of the resazurin test.

3. Milks from diseased udders and milks from physiologically abnormal cows have significant effects on the reduction of resazurin, and hence the test aids in their detection.

4. By observing the rate of color change of resazurin-milk mixtures over a period of hours of incubation, considerable information as to the flora can be obtained.

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# THE BACTERIOLOGY OF SWISS CHEESE

## IV. EFFECT OF TEMPERATURE UPON BACTERIAL ACTIVITY AND DRAINAGE IN THE PRESS

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In the manufacture of Swiss cheese, the heating of the kettle contents to 53° C. or slightly higher, and the high temperature maintained for a number of hours in the press, are known to be essential not only for the proper expulsion of whey from the curd, as pointed out by Orla-Jensen (10), Vas (12), and Koestler (9), but also for the control of bacterial activity. Burri (3) stated that only the thermo-stable organisms survive the heating, with a result that in the press 99 per cent of the surviving lactic acid organisms are either *Streptococcus thermophilus* or *Thermobacterium helveticum*. In a previous paper (6) it was shown that the aerobic gas-forming organisms which are frequently present in milk grew very little if at all in the cheese kettle unless present in large numbers, and that while *Streptococcus lactis* grew early in the kettle it was stopped by the heating process. Of the starter bacteria, *S. thermophilus* (C<sub>3</sub>) grew during the early part of the cooking period; the lactobacilli (39a and Ga) not only did not grow but actually decreased in numbers by dipping time.

A study of the growth of the starter bacteria in the press as reported in a previous paper (7) showed that *S. thermophilus* (C<sub>3</sub>) resumed active growth within 3 or 4 hours after dipping and continued for 6 to 8 hours before any retardation became evident. *Lactobacillus bulgaricus* (Ga) and *Lactobacillus helveticus* (39a) did not begin to grow until 5 to 10 hours after dipping, the time depending on the culture; the Ga culture, with the higher maximum growing temperature, started to grow earlier in the cheese. This indicated that the starter organisms do not begin to grow until temperature conditions are favorable.

Further observations on bacterial activity in the cheese showed certain significant facts. It was found that at 3 hours after dipping the pH of the interior of the cheese (4 or more inches from the hoop edge) was usually between 5.90 and 6.15, while the pH near the outside of the cheese ( $\frac{1}{2}$  to 1 inch beneath the hoop edge) was between 5.60 and 5.85. The more rapid acid production near the hoop edge appeared to be consistent with results reported by Orla-Jensen (11), who showed that whey obtained from the cheese board 3 hours after dipping contained 3 to 4 times as much titratable acid as that obtained from the center of the cheese. His data showed that much of this acid came from used cheese cloths. The higher titratable

acidity at the surface, he believed, was also partially the result of rapid evaporation from the surface of the cheese, although he indicated that other factors may also contribute to this difference. Temperature readings at the center of the cheese were included in these data but temperatures near the periphery were not determined.

In this paper are shown the changes in temperature, bacterial population and pH values which occur at varying distances from the hoop edge toward the center of Swiss cheese during the first 21 hours in the press; data are presented showing the effect of variations in room temperature upon the above factors, the relationship between the production of acid and the expulsion of whey from the cheese, and the effect of these factors upon the quality of the cheese. The bacterial content and acidity of cheese cloths are recorded, and the possible effect of contamination from this source upon the rate of acid production just beneath the periphery of the cheese is discussed.

#### EXPERIMENTAL PROCEDURE

The experimental work was conducted in part at a factory in Ohio, with cheeses averaging 160 pounds, green weight; more detailed studies were made with laboratory cheeses of about 55 pounds, green weight.

The starters used consisted of pure cultures of *S. thermophilus* (C<sub>1</sub>) in combination with pure cultures of either *L. bulgaricus* (Ga or 39aH) or *L. helveticus* (39a), each being grown separately in skim milk or in whey.

The lactobacillus cultures referred to herein have been in use for a number of years in these laboratories and in the factories, and they differ distinctly one from another in morphology, maximum growing temperature and the amount of acid produced in milk; however, the identities of these cultures have not been positively established.

Experience has shown that the medium used for the preparation of starters in factories often is not sterilized adequately, with the result that the starters often are not pure cultures. In these experiments particular care was taken to insure the sterilization of the skim milk or whey used in the preparation of starters and every precaution was taken to prevent subsequent contamination or the use of mixed cultures.

Temperature readings were obtained by inserting standardized thermometers so that the mercury bulbs would be located at the points at which temperatures were to be determined. In making readings at successive 1-inch distances from the hoop edge, a cardboard strip with  $\frac{1}{4}$ -inch holes 1 inch apart was placed on the top of the cheese along its diameter, the first hole being 1 inch from the circumference. The thermometers were inserted through the holes in the strip and pushed into the cheese until their bulbs were at points equidistant from the top and bottom surfaces.

Samples of cheese for pH, moisture, and bacterial count determinations were obtained by removing a 6-inch plug, by means of a trier, from the hoop

side of the cheese halfway between the top and bottom surfaces. The plug was cut cleanly into 1-inch sections, measuring from the hoop end of the plug. Then  $\frac{1}{4}$  inch was cut off from both ends of each section, leaving the middle  $\frac{1}{2}$  inch. This was cut into two halves longitudinally. One part was used for the pH determination and the other for the bacterial count. A section from the inner end of the plug, more than 4 inches from the hoop edge, was used for the duplicate moisture determination. The sample used for bacterial counts was ground in a sterile mortar with sodium citrate and water, by the method described by Burkey (1). The direct microscopic count was made from this homogenous sample.

The cooking temperature used in the manufacture of cheese was in all cases  $53^{\circ}\text{C}$ .

### RESULTS

The results of temperature determinations, recorded in Table 1, show that the outer area of the cheese cools rapidly and that a decrease of  $2^{\circ}$  to  $4^{\circ}\text{C}$ . in room temperature results not only in an acceleration of cooling just inside the surface of the cheese during the first 3 or 4 hours but also a correspondingly more rapid cooling in the interior, especially after that time. When the room temperature is low, the temperatures of all parts of the cheese are likewise relatively low at 21 hours; but the difference between that of the interior and that of the outer area is practically the same as when the room temperature is high. In securing the data shown in Table 1, in which are shown average results in 7 factory cheeses made on

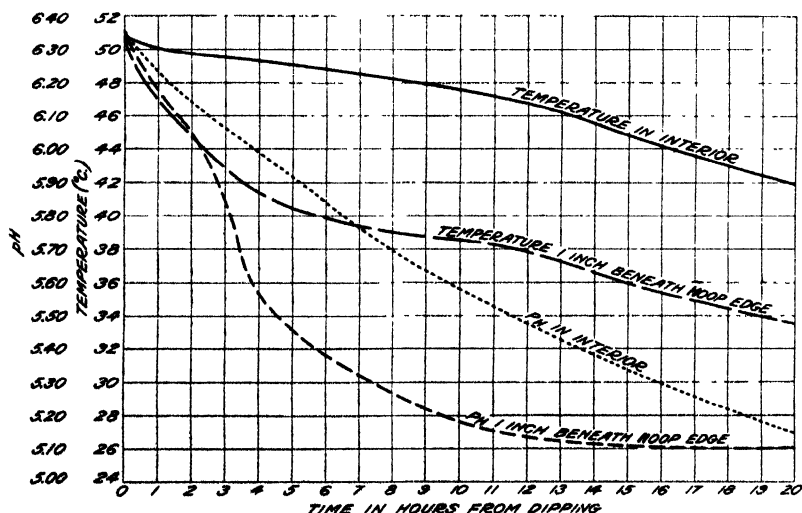


FIG. 1. TEMPERATURE AND pH CURVES FOR THE INTERIOR AND OUTER 1 INCH OF A FACTORY CHEESE.



three different days, the room temperatures were recorded at frequent time intervals, and also the temperatures at the following points within the cheeses: at the central point in the interior; 1 inch beneath the rind at the center of the top surface; and 1 inch beneath the rind at the hoop edge, halfway between the top and bottom surfaces.

The typical relationship between the decrease in temperature at different points in Swiss cheese and the development of acidity at these points is shown in Figure 1. These data are from a factory cheese made with  $\frac{1}{8}$  per cent of *L. bulgaricus* (Ga) skim milk starter grown at 42.5° C. and  $\frac{1}{8}$  per cent of *S. thermophilus* (C<sub>a</sub>) whey starter grown at 37°. It will be noted that a rapid decrease in temperature at the hoop edge results in a correspondingly rapid development of acidity.

More detailed results were obtained in a study made of a 55-pound laboratory cheese. The pH, bacterial count and temperature determinations were made at regular intervals of time at successive 1-inch distances from the hoop edge. These data are shown in Table 2. It will be noted that in general the increase in numbers of the starter organisms (C<sub>3</sub> and Ga) and the increased acid production occur as a result of the decrease in temperature at that point in the cheese. C<sub>3</sub> increased rapidly in numbers in the area 1 inch beneath the hoop edge but growth, as shown by increase in numbers, did not occur until several hours later in the interior. (Ga increased in numbers in the area 1 inch beneath the hoop edge 1 to 3 hours after

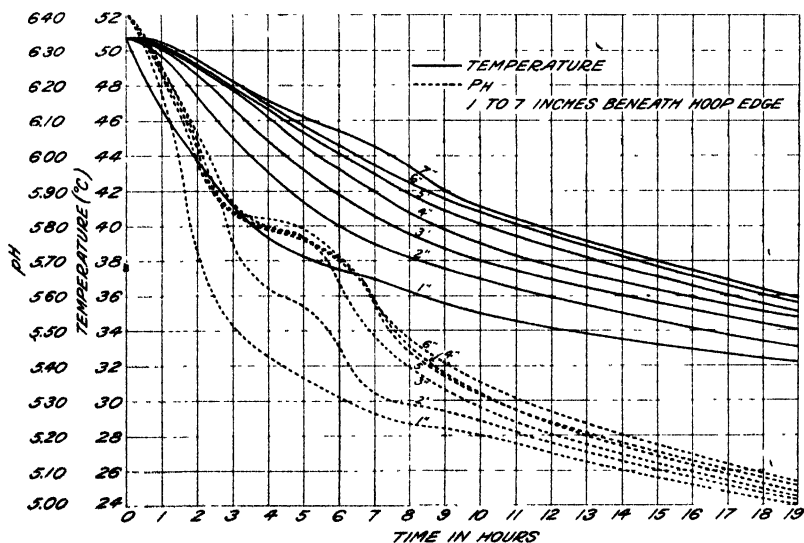


FIG. 2. TEMPERATURE AND pH CURVES AT 1-INCH DISTANCES FROM THE HOOP EDGE OF A LABORATORY CHEESE.

TABLE 2  
Temperature, bacterial growth, and acidity at different points in laboratory Swiss cheese (*L. bulgaricus* and *S. thermophilus* starters)

TIME	ROOM TEMP.	1 INCH			2 INCHES			3 INCHES		
		Temp.	H ion Conc.	Organ-ism	Count	Temp.	H ion Conc.	Organ-ism	Count	Count
hrs.	°C.	°C.	pH		per gram	°C.	pH		per gram	per gram
0	29.7									
1	29.0	46.7	6.18	C <sub>1</sub>	250,000,000	49.7	6.23	C <sub>2</sub>	104,000,000	91,600,000
3	27.1	41.0	5.51	Ga	1,320,000	45.0	5.74	Ga	198,000	1,190,000
5	29.8	38.2	5.37	C <sub>1</sub>	663,000,000	41.3	5.57	C <sub>2</sub>	161,000,000	111,000,000
7	30.8	36.9	5.26	Ga	25,500,000	39.0	5.32	Ga	5,000,000	7,500,000
9	29.8	35.5	5.22	C <sub>1</sub>	873,000,000	37.5	5.27	C <sub>2</sub>	154,000,000	124,000,000
19		32.2	5.00	Ga	278,000,000	33.0		Ga	73,800,000	20,500,000
					1,580,000,000				425,000,000	174,000,000
					371,000,000				462,000,000	322,000,000
					923,000,000				398,000,000	599,000,000
					269,000,000				437,000,000	

TABLE 2—(Continued)

TIME	ROOM TEMP.	4 INCHES			5 INCHES			6 INCHES		
		Temp.	H ion Conc.	Organ-ism	Count	Temp.	H ion Conc.	Organ-ism	Count	Count
hrs.	°C.	°C.	pH		per gram	°C.	pH		per gram	per gram
0	29.7	50.7	6.41	C <sub>1</sub>	116,000,000	50.3	6.25	C <sub>2</sub>	60,300,000	99,500,000
1	29.0	50.1	6.24	Ga	1,300,000	47.9	5.84	Ga	207,000	3,590,000
3	27.1	47.8	5.94	C <sub>1</sub>	143,000,000	45.4	5.76	C <sub>2</sub>	119,000,000	92,700,000
5	29.8	44.5	5.79	Ga	105,000,000	43.0	5.60	Ga	63,600,000	2,330,000
7	30.8	42.1	5.59	C <sub>1</sub>	198,000	40.8	5.37	C <sub>2</sub>	1,980,000	76,500,000
9	29.8	39.8	5.38	Ga	83,300,000	35.0	5.07	Ga	139,000,000	3,030,000
19		34.7			118,000,000				192,000,000	140,000,000
					142,000,000				156,000,000	89,500,000
					161,000,000				502,000,000	168,000,000
					246,000,000					380,000,000
					787,000,000					

dipping; in the 2-inch area in 5 hours, and at 3, 4, 5, and 6 inches no increase in numbers was evident until the sixth or seventh hour after dipping. The temperature and pH curves for the same cheese, presented in Figure 2, demonstrate the rapid decrease in temperature in the outer 1-inch area, the progressively less rapid decrease at increasing distances from the hoop edge, and the corresponding increase in acid with the decrease in temperature. It may be seen from these results that a sample to be representative of the interior of the cheese should be obtained at a point at least 4 inches from the hoop edge. Each hump in the pH curves in Figure 2 represents a diminution of acidity which may occur when the growth of  $C_3$  is retarded before the lactobacilli begin to grow.

Data from a similar study of laboratory cheeses made with a different lactobacillus culture are shown in Table 3. The starters used were *L. hel-*

TABLE 3  
*Bacterial growth and acidity in laboratory Swiss cheese (L. helveticus and S. thermophilus starters)*

NO.	HOURS AFTER DIPPING	1 INCH BENEATH HOOP EDGE			INTERIOR OF CHEESE		
		pH	Counts per gram		pH	Counts per gram	
			39a	$C_3$		39a	$C_3$
1	1	6.29			6.36	5,440,000	39,700,000
	3	5.56	5,430,000	888,000,000	6.07	6,940,000	38,900,000
	5	5.28	48,700,000	849,000,000	5.82	2,950,000	207,000,000
	7	5.21	193,000,000	1,410,000,000	5.44	1,200,000	362,000,000
	9	5.15	355,000,000	1,380,000,000	5.39	3,050,000	453,000,000
	21	4.98			5.00	367,000,000	667,000,000
2	7	5.23	245,000,000	790,000,000	5.48	2,650,000	273,000,000
	11	5.01	533,000,000	1,200,000,000	5.36	63,200,000	158,000,000

*veticus* (Bergey, 1934) (39a) and *S. thermophilus* ( $C_3$ ). These results further demonstrate the early growth of  $C_3$  throughout the cheese. 39a began to grow about 3 hours after dipping in the area 1 inch from the hoop edge, but there was no increase in numbers in the interior of the cheese until the ninth hour.

The effect of combinations of starters of different activities on bacterial growth and pH in the outer 1-inch area and in the interior of factory cheese is shown in Table 4. The starters used in the 3 cheeses were: No. 1,  $\frac{1}{12}$  per cent of *L. bulgaricus* (39aH) skim milk grown at 46° C. for 12 hours and  $\frac{1}{2}$  per cent of  $C_3$  whey grown at 36.5° for 12 hours; No. 2,  $\frac{1}{12}$  per cent of 39aH whey grown at 43° for 12 hours and  $\frac{1}{2}$  per cent of  $C_3$  whey grown at 38.5° for 12 hours; No. 3,  $\frac{1}{12}$  per cent of 39aH whey grown at 43° for 12 hours and  $\frac{1}{2}$  per cent of  $C_3$  skim milk grown at 36.5° for 12 hours.

The data show that  $C_3$  and 39aH are more active in the cheese when grown in skim milk than when grown in whey.

TABLE 4  
*The effect of L. bulgaricus (39aH) and S. thermophilus (C<sub>3</sub>) starters of different activities on bacterial growth, acidity and final results in factory Swiss cheese*

CHEESE NO.	KIND OF STARTERS	HOURS AFTER DIPPING	1 INCH BENEATH HOOF EDGE				INTERIOR OF CHEESE				DRAINING APPEARANCE 21 HOURS	FINAL SCORE
			Counts per gram		pH	Counts per gram		pH				
			39aH	C <sub>3</sub>		39aH	C <sub>3</sub>					
1	39aH in skim milk C <sub>3</sub> in whey	0			6.34	422,500	9,362,000	6.34	Fairly dry; slightly rubbery	82		
		1¼	35,000	21,056,000	5.91	227,050	12,301,000	6.15				
		3½	28,160,000	429,000,000	5.50	1,500,000	124,000,000	5.90				
		10	1,433,600,000	538,200,000	5.04	444,500,000	161,290,000	5.65				
		22						5.20				
2	39aH in whey C <sub>3</sub> in whey	0			6.39	173,250	17,136,000	6.39	Slightly wet; leaky, short	78		
		1¼	35,000	21,350,000	6.04	136,000	20,336,000	6.23				
		3½	6,200,000	566,000,000	5.58	1,500,000	96,000,000	6.05				
		10	481,650,000	760,300,000	5.32	369,180,000	245,700,000	5.55				
		22						5.33				
3	39aH in whey C <sub>3</sub> in skim milk	0			6.33	176,000	23,296,000	6.33	Driest; rubbery, more firm	95½		
		1¼	34,100	34,100,000	5.82	102,300	40,000,000	5.87				
		3½	20,480,000	395,520,000	5.50	1,500,000	238,000,000	5.60				
		10	390,610,000	139,190,000	5.08	172,720,000	301,625,000	5.52				
		22						5.10				

In this experiment the rapid acid production in the interior of cheese No. 3, especially during the first 3 hours, resulted in more extensive drainage and in a higher scoring cheese than was obtained in cheeses Nos. 1 and 2. This would indicate that the basis for proper drainage is established during the first few hours after dipping. It is evident that *S. thermophilus* (C<sub>1</sub>), which is the active starter during this period, performs a significant rôle in the drainage of Swiss cheese (2). Furthermore, these results indicate that the proper combination of starters to use depends upon the activity of each starter. The more active the lactobacillus starter the greater is the need for an active *S. thermophilus* starter to facilitate drainage from the interior of the cheese.

A great difference in the rate of cooling between the interior and the outer 1-inch area causes a correspondingly great difference in the rate of acid production and thus causes expulsion of a small proportion of the whey. In 60 laboratory cheeses there were 16 cheeses in which the difference in pH at 3 hours between the interior and the area 1 inch beneath the hoop edge was less than 0.30, with an average percentage of moisture in 21 hours of 38.02; 21 cheeses with a difference in pH of 0.30 to 0.40, with an average percentage of moisture of 38.39; and 23 cheeses with a difference in pH of over 0.40, with an average percentage of moisture of 38.58. The drier cheeses had the higher average scores.

In these 60 cheeses, determinations of moisture both in the interior and in the area 1 inch beneath the hoop edge were made at 21 hours. In 48 of the 60 cheeses, the percentage of moisture was greater just under the rind than in the interior. This tendency for moisture to collect just beneath the surface is a possible explanation for the occurrence of the defective whitish, grainy, and checked area that is often found just beneath the rind.

#### ACIDITY AND BACTERIAL COUNTS OF CHEESE CLOTHS

In connection with studies of acidity it was found that at dipping the pH of the whey on the top of the cheese and in contact with the dipping cloth was 6.32, while that of the whey obtained from within the cheese was 6.45. Orla-Jensen (11), in work referred to earlier in this paper, found that acidity in used cheese cloths materially increased the titration values of the whey as it flowed from the cheese boards. It was therefore considered desirable to study the effect of the cloths upon bacterial activity in the cheese.

In order to study the amounts of acid contained in dipping cloths, 2 cloths were each soaked for 10 minutes in 1,500 cc. of sterile distilled water. The rinse-waters thus obtained, when titrated with phenolphthalein as an indicator, were found to contain 0.078 and 0.065 per cent acid, respectively.

In order to determine whether the bacteria present in the cloths used on the cheese had any influence on the rate of acid development at the sur-

face of the cheese, two cloths were used on cheeses and then washed as usual; one was dried in the usual manner after washing, and the other was thoroughly autoclaved before drying. On the following day these cloths were each soaked in 500 cc. of sterile distilled water for 10 minutes. All but a small amount of this water was absorbed by the cloths. In the remaining water the counts of organisms per cc. were as follows: (1) from unsterilized cloth: 1,000 yeasts, 10,000,000 streptococci, and 100,000 rods; (2) from autoclaved cloth: 10 yeasts, 1,000 streptococci, and 10 rods. The unsterilized or regular cheese cloth contained large numbers of bacteria, particularly of the *S. thermophilus* type, as compared with the low counts in the autoclaved cloth. These two cloths were used on two factory cheeses at the second turning. No difference was found in the rates of acid development at the surfaces of the two cheeses.

#### DISCUSSION

The temperature of Swiss cheese in the press has been found to decrease rapidly in the outer 1-inch area during the first 3 or 4 hours after dipping. The rate of cooling becomes less as the distance from the hoop edge toward the center becomes greater. After the fifth hour all parts of the cheese cool at a fairly uniform rate, the temperature reaching 36° to 46° C. in the interior of the cheese in 21 hours. The decrease in temperature is comparatively large in small cheese and in those held at a low room temperature.

The rapid cooling in the outer 1-inch area, as compared with that in the interior, results in a corresponding increase in bacterial growth and acid production near the surface. When the difference in pH at 3 hours between the outer area and the interior is large, *e.g.*, more than 0.30, the cheese tends to retain moisture and is therefore likely to show checking near the rind or a splitting of the curd known as "glass."

Experimental data presented herein show that acid production, as shown also in paper No. II of this series (7), and the extent of drainage in the interior of the cheese during the first 4 to 6 hours, are due largely to the growth of *S. thermophilus*. The lactobacilli do not start to grow in the interior of the cheese until 5 to 10 hours after dipping, when the temperature conditions become favorable. *L. bulgaricus* (39aH and Ga) having a higher maximum growing temperature begins to grow in the interior of the cheese at about the fifth or sixth hour, but *L. helveticus* (39a) does not begin to grow until the ninth hour or later.

In the outer 1-inch area, however, *S. thermophilus* begins to grow soon after the cheese is placed in the press; Ga and 39aH about an hour later; and 39a about 3 hours after dipping. Consequently, there is a tendency for acid production to start quickly and proceed rapidly near the periphery. The increase in acidity hastens the expulsion of whey from the cheese particles, causing them to cohere more closely and finally to mat together. As a result, the more rapidly acid is produced near the periphery, the sooner there

is formed a matting of the cheese particles, causing in a few hours the gradual formation of a layer about the cheese which, as time goes on, becomes an almost impervious barrier through which whey can pass only very slowly. If, therefore, the lactobacilli are very active and *S. thermophilus* weak or low in numbers, an excessive amount of whey is retained and it tends to collect near the rind. This causes the cheese to be wet and to "leak" in the press, resulting finally in the defects referred to above. When, therefore, an active Ga or 39aH starter is used, a particularly active *S. thermophilus* starter should be employed to hasten drainage from the interior of the cheese. The use of a lactobacillus starter such as 39a, which produces delayed activity in the cheese, requires the use of a *S. thermophilus* starter which will produce prolonged activity so that the production of acid will be continued until the lactobacilli begin to grow.

It has been pointed out (5) that *S. thermophilus* probably always occurred naturally in the starter or has had its source either in the milk or cheese equipment or in the milk itself. Reports of investigators in Switzerland show the presence of a streptococcus in the natural rennet from stomachs. Our observations, based on several direct isolations, have shown that *S. thermophilus* is often present as a contamination of the lactobacillus cultures as handled in the cheese factories. Due to its relatively high thermal death point, *S. thermophilus* withstands the usual washing and sterilizing of cheese equipment. The equipment is invariably heavily seeded with this organism, which may thus gain entrance into the cheese day after day. An examination of the fresh milk also shows the presence of this organism, especially if whey is returned to the farmers in milk cans. The contamination by *S. thermophilus* from these sources may be so great that it may be necessary to add only a small amount of the streptococcus starter.

The rate at which drainage occurs must also be given careful consideration. Slow biological activity in the interior results in slow drainage of whey from the cheese and provides conditions favorable for the growth of gas-forming bacteria, which if present in large numbers may cause the pressler defect, or an excessive amount of acid may be formed later in the press and as a result, in the early stages of ripening, normal eye-formation may be retarded and the cheese may be "blind" or the defects known as "glass" and rind-checking may occur. If acid production and drainage progress too rapidly during the early hours on the press (pH 5.75 or less at 3 hours), the further production of acid is likely to be deficient (pH 5.30 or more at 21 hours), and the cheese body is likely to be short and crumbly and the curd poorly knit, as pointed out in papers II (7) and III (8) of this series. Such a cheese tends to take on "set" too early and to become "overset," and the eyes are likely to be "cabbagy" and irregular in shape. Koestler (9) has pointed out the harmful effects of too little and too much acidity in the press, from the standpoint of effects upon curd properties and upon the ripening and the final quality. Dorner (4) has concluded

that it is desirable to so regulate the use of starters as to provide a rate of acid production which is neither too rapid nor too slow, thereby promoting adequate drainage and improved quality of cheese.

The drainage of Swiss cheese results in the loss of other substances in addition to moisture. These substances undoubtedly include such readily available food for bacterial growth as lactose, soluble proteins and certain colloidal nitrogenous material. The amounts of these substances retained in the cheese undoubtedly depend in part on the rate and amount of drainage. If drainage progresses too rapidly there is apt to be a deficiency of these food materials for the proper growth of the starter and eye-forming bacteria. At the same time other bacteria, due to their less fastidious natures, can grow and may cause abnormal eye-formation and the other defects referred to above. On the other hand, if drainage is retarded, larger amounts of these food materials are apt to be retained, causing too great bacterial activity. In this case the excessive amount of acid formed is likely to retard eye-formation and influence the normal ripening process unfavorably. Additional work is needed to show what effect drainage has upon eye-formation and upon the final ripening of Swiss cheese.

The proper use of starters will provide sufficient numbers of *S. thermophilus* of the proper activity to reduce the pH in the interior to about 6.00 in 3 hours, as shown in papers II (7) and III (8) of this series, or a pH decrease of 0.30 to 0.40 in that time, and will provide sufficient numbers of active lactobacilli to continue the decrease in pH at a fairly uniform rate so that the pH may reach 5.00 to 5.15 at 21 hours. This 21-hour pH reading may tend to be misleading the nearer it approaches 5.00 or slightly below, since this is about the maximum acidity reached in Swiss cheese. In order to be certain of a uniform decline in pH, it is believed desirable either to make a pH reading in 16 to 18 hours after dipping or to work toward a pH value of 5.10 to 5.15 at 21 hours.

#### SUMMARY

Temperature, pH, and bacterial counts were determined in Swiss cheese in the press at regular time intervals from dipping to 21 hours in samples taken at definite distances from the outer edge of the cheese. Results were obtained from studies of 55-pound laboratory cheeses and also from large factory cheeses. Moisture determinations were made on samples from the laboratory cheeses. The following conclusions were drawn from the results:

1. The area just beneath the rind cools more rapidly than the interior.
2. Bacterial growth and acid production correspond in general with the decrease in temperature of each part of the cheese.
3. While all the starter bacteria begin to increase in numbers in the area 1 inch beneath the rind during the first few hours after dipping, the initiation of growth in the interior was shown to occur at the following intervals of time from dipping: *S. thermophilus* ( $C_3$ ), within 2 or 3 hours;

*L. bulgaricus* (Ga and 39aII), after 5 or 6 hours; and *L. helveticus* (39a), after 9 or 10 hours.

4. Essential functions of *S. thermophilus* in Swiss cheese in the press are the production of acid and the consequent facilitation of drainage from the interior of the cheese during the first 4 to 6 hours after dipping.

5. Large differences in pH between the interior and the area just beneath the rind result in insufficient drainage and high moisture content, and may tend to cause such defects as checking near the rind or a splitting of the curd known as "glass."

6. Bacterial content of cheese cloths was found not to influence the rate of acid production near the surface of the cheese.

7. Samples from the interior of the cheese, in order to yield results representative of the interior of the cheese, should be obtained at points at least 3 or 4 inches from the hoop edge.

8. A judicious use of starters promotes sufficient acid production throughout the cheese and thereby aids in uniform drainage.

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# COMBINATION OF CATALYSTS TO REDUCE DIGESTION TIME IN THE DETERMINATION OF NITROGEN

## II. DAIRY PRODUCTS

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### INTRODUCTION AND HISTORICAL REVIEW

The time required for the determination of nitrogen has been materially reduced since Kjeldahl (1) first proposed his original method. This reduction has been made possible by introducing into the reaction mixture potassium sulphate and catalysts such as mercury and copper. Just recently, the use of selenium has been advocated as a catalyst. Although Lauro (2) was the first to investigate the use of selenium, this substance has been combined with other catalysts by several investigators (3 to 11 inc.) Of these workers, several (7, 8, 10) have found this element to reduce materially the amount of nitrogen obtained in the analysis. Sandstedt (11) also has found with selenium some loss of nitrogen on long heating.

Nearly all of the investigations of the determination of nitrogen in which selenium has been used have been with cereal products. In this communication, there are reported the results obtained when selenium is used with other catalysts in the determination of nitrogen in dairy products. No experimental data are available on the efficiency of selenium when combined with copper, mercury, and strong hydrogen peroxide.

### METHODS AND EXPERIMENTAL DATA

The method employed was the Gunning modification of the original Kjeldahl method, which is official with the Association of Official Agricultural Chemists (12). Given amounts of each dairy product were weighed or measured into ten digestion flasks. In case of cheese and milk powder, one gram was used. With the liquid products, the weight was determined by multiplying the cubic centimeters by the specific gravity. Five cc. of evaporated milk and ten cc. of fresh milk were used. After the dairy product had been added to the digestion flask, ten grams of nitrogen-free potassium sulphate and twenty cc. of concentrated sulphuric acid were added. The following catalysts or combination of catalysts were separately added to each of the nine digestion flasks, the tenth being used as a control without catalysts.

(1) One cc. of thirty per cent hydrogen peroxide was added at the beginning of the heating process and then each five minutes during the digestion until the solution cleared.

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TABLE 1  
*Determination of proteins in dried milk*

CATALYST	SKIMMED MILK POWDER		PROTEIN MILK POWDER		LACTIC ACID SKIMMED MILK		WHOLE LACTIC ACID MILK POWDER		WHOLE MILK POWDER		AVERAGE	
	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein
H <sub>2</sub> O <sub>2</sub> . . . . .	47	36.14	61	35.73	53	34.99	39	24.62	84	23.16	56.8	30.93
HgO . . . . .	25	35.73	30	35.34	26	35.65	20	24.81	22	23.93	24.6	31.09
HgO + H <sub>2</sub> O <sub>2</sub> . . . . .	24	35.70	32	35.15	21	35.15	16	24.57	24	24.14	23.4	30.94
Se. . . . .	27	35.73	31	35.22	23	35.28	26	24.86	28	23.78	27.0	30.97
Se. + H <sub>2</sub> O <sub>2</sub> . . . . .	28	35.86	27	35.41	20	35.48	20	24.98	20	24.20	23.0	31.19
CuSO <sub>4</sub> . . . . .	38	35.80	24	34.77	27	35.18	22	24.90	22	23.99	26.6	30.93
CuSO <sub>4</sub> + H <sub>2</sub> O <sub>2</sub> . . . . .	29	35.78	25	34.97	29	34.97	17	24.52	23	23.78	24.6	30.80
HgO + Se. + CuSO <sub>4</sub> . . . . .	13	35.86	12	35.34	10	35.22	11	24.55	13	24.08	11.8	31.01
HgO + Se. + CuSO <sub>4</sub> + H <sub>2</sub> O <sub>2</sub> . . . . .	11	35.93	10	35.41	9	34.97	12	25.06	8	23.80	10.0	31.03
Control . . . . .	97	35.43	92	34.83	129	35.28	75	24.81	101	24.20	98.8	30.91
Average . . . . .		35.80		35.22		35.22		24.77		23.91		30.98

TABLE 2  
*Determination of proteins in fluid milk*

CATALYST	WHOLE MILK NO. 1		WHOLE MILK NO. 2		WHOLE MILK NO. 3		WHOLE MILK NO. 4		BUTTER MILK		SKIMMED MILK		AVERAGE	
	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein
H <sub>2</sub> O <sub>2</sub> ....	43	3.39	65	3.73	45	3.39	47	3.45	30	3.09	42	3.59	45.3	3.44
HgO <sub>2</sub> .....	26	3.48	28	3.74	21	3.48	27	3.54	16	3.03	18	3.68	22.7	3.49
HgO + H <sub>2</sub> O <sub>2</sub>	20	3.49	26	3.76	18	3.47	24	3.50	13	3.04	15	3.67	19.3	3.49
Se. ....	24	3.38	28	3.71	21	3.36	24	3.47	21	3.01	26	3.69	24.0	3.44
Se. + H <sub>2</sub> O <sub>2</sub>	20	3.45	21	3.69	21	3.42	22	3.49	20	3.05	20	3.66	20.7	3.46
CuSO <sub>4</sub>	23	3.38	27	3.64	24	3.40	20	3.45	17	2.98	22	3.62	22.2	3.41
CuSO <sub>4</sub> + H <sub>2</sub> O <sub>2</sub>	20	3.36	19	3.75	20	3.43	21	3.42	18	2.96	19	3.64	19.5	3.43
HgO + Se. + CuSO <sub>4</sub>	12	3.48	14	3.71	14	3.48	17	3.48	11	3.03	14	3.77	13.7	3.49
HgO + Se. + CuSO <sub>4</sub> + H <sub>2</sub> O <sub>2</sub>	11	3.49	12	3.75	13	3.46	15	3.47	8	3.01	10	3.72	11.5	3.48
Control	119	3.44	118	3.74	119	3.42	169	3.50	90	2.97	151	3.71	127.7	3.46
Average		3.43		3.72		3.43		3.48		3.02		3.63		3.46

TABLE 3  
*Determination of proteins in evaporated and condensed milk*

CATALYST	EVAPORATE MILK NO. 1		EVAPORATE MILK NO. 2		EVAPORATE MILK NO. 3		EVAPORATE MILK NO. 4		EVAPORATE MILK NO. 5		CONDENSED MILK NO. 1		AVERAGE	
	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein
H <sub>2</sub> O <sub>2</sub>	45	6.66	42	6.57	48	7.02	43	7.02	55	7.21	70	7.85	50.6	7.06
HgO	26	6.81	27	6.48	28	7.08	28	7.08	23	7.08	20	8.10	25.3	7.11
HgO + H <sub>2</sub> O <sub>2</sub>	22	6.79	23	6.63	23	7.22	28	7.21	19	7.21	21	7.97	22.7	7.17
Se.	24	6.82	28	6.57	31	7.08	26	7.02	26	7.14	29	7.91	27.3	7.09
Se. + H <sub>2</sub> O <sub>2</sub>	22	6.73	27	6.63	29	7.04	24	7.08	26	7.21	26	7.94	25.7	7.11
CuSO <sub>4</sub>	19	6.55	30	6.57	27	7.02	23	6.96	26	7.20	27	7.68	25.3	7.00
CuSO <sub>4</sub> + H <sub>2</sub> O <sub>2</sub>	20	6.74	24	6.59	27	7.08	26	7.02	21	7.08	26	7.72	24.0	7.04
HgO + Se. + CuSO <sub>4</sub>	14	6.71	16	6.63	16	7.22	14	7.08	10	7.12	16	7.91	14.3	7.11
CuSO <sub>4</sub> + H <sub>2</sub> O <sub>2</sub>	11	6.63	10	6.63	13	7.27	11	7.02	9	7.14	13	7.78	11.2	7.08
Control	89	6.72	92	6.57	90	7.22	101	7.14	95	7.14	61	7.85	88.0	7.11
Average		6.72		6.59		7.13		7.06		7.15		7.87		7.09

TABLE 4  
*Determination of proteins in cheese*

CATALYST	LEGHORN		BRICK		HOLLAND		SWISS		AMERICAN CREAM		ROQUEFORT		AVERAGE	
	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein
H <sub>2</sub> O <sub>2</sub>	46	28.80	50	27.60	54	30.10	60	27.63	56	28.77	47	14.36	52.2	26.21
HgO	28	28.77	18	27.31	29	29.98	25	27.29	33	28.45	29	14.55	27.0	26.06
HgO + H <sub>2</sub> O <sub>2</sub>	30	29.47	19	27.33	26	30.32	20	27.33	30	28.71	24	14.39	24.8	26.26
Se.	24	28.90	23	27.60	29	29.86	23	27.00	38	28.37	29	14.23	27.7	26.03
Se. + H <sub>2</sub> O <sub>2</sub>	24	29.03	23	27.33	27	29.92	21	27.29	34	28.77	25	14.23	25.7	26.10
CuSO <sub>4</sub>	28	28.96	29	26.52	27	30.30	24	27.85	26	28.20	26	14.04	26.7	25.98
CuSO <sub>4</sub> + H <sub>2</sub> O <sub>2</sub>	24	28.64	27	27.31	25	29.98	19	27.33	25	28.71	25	14.20	24.2	26.03
HgO + Se. + CuSO <sub>4</sub>	15	28.95	14	27.60	12	30.31	9	27.60	10	28.74	13	14.06	12.2	26.21
HgO + Se. + CuSO <sub>4</sub> + H <sub>2</sub> O <sub>2</sub>	16	29.09	10	27.31	9	30.05	7	27.85	8	28.71	10	14.02	10.0	26.17
Control . . . .	87	29.47	103	26.55	135	30.17	94	27.63	123	27.98	86	14.29	104.7	26.02
Average		29.01		27.25		30.10		27.48		28.56		14.24		26.11

- (2) Five-tenths gram of  $\text{HgO}$ .
- (3) Five-tenths gram of  $\text{HgO}$  with hydrogen peroxide added as in (1).
- (4) Two-tenths gram of selenium.
- (5) Two-tenths gram of selenium with hydrogen peroxide added as in (1).
- (6) One gram  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ .
- (7) One gram  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  with hydrogen peroxide added as in (1).
- (8) Three-tenths gram  $\text{HgO}$ , 0.1 gram selenium, and 0.5 gm.  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ .
- (9) The same catalysts as in (8) with hydrogen peroxide added as in (1).

The contents of each flask were digested over electrically-heated plates, all units of which were of the same construction and gave the same amount of heat. Each sample was heated until the liquid cleared.

The records of the digestion times and the amount of proteins obtained are given for each dairy product in Tables 1, 2, 3, and 4.

A study of the Tables shows that when strong hydrogen peroxide was used with  $\text{CuSO}_4$ ,  $\text{HgO}$ , and selenium separately, there was only a slight reduction in the digestion time over that obtained without the hydrogen peroxide. The accuracy of the protein determination was not affected. The combination of copper, mercury, and selenium materially reduced the time of digestion over that obtained when each catalyst was used separately. The addition of strong hydrogen peroxide to the mixture of these three catalysts still further reduced the time of digestion, but not enough to warrant the additional trouble and expense.

In every case where  $\text{CuSO}_4$  was used, either alone or with hydrogen peroxide, the results for protein seem to be slightly lower than those obtained with other catalysts.

#### CONCLUSIONS

1. Hydrogen peroxide when used with copper, mercury, and selenium (separately or in combination) reduces somewhat the digestion time and does not impair the accuracy of the determination.

2. The combination of the three catalysts ( $\text{Cu}$ ,  $\text{Hg}$ , and  $\text{Se}$ ) showed a reduction in digestion time from 105 to 13 minutes (averages) when compared to that of the Gunning method. When hydrogen peroxide was used with these three catalysts, the reduction in time was from 105 to 10.7 minutes (averages).

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# THE RELATION OF MASTITIS TO RENNET COAGULABILITY AND CURD STRENGTH OF MILK\*

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## INTRODUCTION

Various estimates have been made as to the prevalence of mastitis among cows that furnish milk to our regular commercial outlets. As early as 1888 Hess, Schaffer and Bondzynski (1) classed udder inflammations with diseases that most commonly afflict domestic animals. Minett (2) in 1930, Seelemann (3) in 1932, and Rosell (4) in 1933 reviewed publications showing the prevalence of mastitis in various sections of the world; citations showing from 15 to 40 per cent of the cows infected with mastitis are common, and in some cases the majority of cows were reported as afflicted. Seelemann points out that the widespread prevalence of mastitis is usually unsuspected. Dairymen are likely to feel that in their respective communities the disease is much less common, but when surveys are made they usually show its prevalence to be in harmony with published results.

While the exact estimates may differ, it is clearly evident that an appreciable portion of our regular commercial milk comes from cows afflicted with subclinical mastitis. Unquestionably many investigations of dairy problems in the past have been complicated by the inclusion of such unsuspected abnormal milk. Fortunately such investigations still are typical of commercial conditions, but we should also understand dairy problems in relation to strictly normal milk so that we may anticipate the benefits or even new problems that may arise from the elimination of milk from mastitis udders. The present study was undertaken to determine the relation of mastitis to the rennet coagulability and curd strength of milk.

## REVIEW OF LITERATURE

In 1932 Monier and Sommer (5) in a paper before the American Dairy Science Association reported that two samples of milk which had been selected and studied as typical soft-curd milk were later found from the veterinarian's record to have shown a history of mastitis during the time the samples were being studied. Further work then undertaken showed that while mastitis was not the sole cause of soft-curd milk, it did lower the curd strength. In 1933 Doan and Welch (6) confirmed this conclusion and attributed the lower curd strength of milk from infected udders mainly to the lower casein content of such milk. In 1934 Hansen, Theophilus,

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Atkeson and Gildow (7) reported results to show that mastitis caused by streptococcic infection invariably lowered the curd strength, but that staphylococcic infections did not affect the curd strength appreciably.

As early as 1888 Hess *et al.* (1) emphasized the damaging effect of milk from infected udders on cheese making. Koestler (8) in 1921 noted the slower coagulation of such milk with rennin, and in 1926 Koestler *et al.* (9) reported a higher protein and fat loss in the whey and a more milky appearing whey in making cheese from such abnormal milk. Hill in 1932 (10) compared soft-curd and hard-curd milk in cheese making; he found a higher fat loss in the whey with the soft-curd milk but there was no significant difference in the casein content of the whey. The protein content of the soft-curd milk was 3.33 per cent as compared with 3.8 per cent in the hard-curd milk.

#### PROCEDURE

The milk samples used in this study were obtained from the cows in the University of Wisconsin Herd which had for some time been segregated into "streptococcus-free" and "infected" groups on the basis of close veterinary supervision which involved udder inspection by manipulation, and on the basis of the following tests applied to the milk: strip cup test, chlorine content by direction titration, brom cresol purple test, catalase test, rennet test, plate colony count on blood agar. Where streptococci were found, tests were made to identify them further, and in all cases the organisms were found to belong to the alpha-group. These tests were in progress throughout this study, and diagnoses were being made entirely independent of it, with the aim of securing a herd that was strictly normal; the "infected" group, as a result, included some cows for which the diagnosis was doubtful. All the cases of mastitis included were comparatively mild; the milk samples in all cases, even where quarter samples were studied, were normal in appearance to casual or untrained observers and in ordinary herd practice would in all probability have been included in the regular milk supply.

The curd strength tests were made according to the Hill (11) procedure, modified as to the method of mixing the milk and coagulant, and as to the equipment used. Mixing was accomplished by discharging the previously tempered milk sample from a 100 cc. pipette with an unusually large delivery opening (water at 20° C., discharge time 4.4 sec.), into the jar that already contained the coagulant, giving the jar a rotary motion in the meanwhile and directing the stream towards the lower corner of the jar. The same curd knife was used for all tests, reversing the blades on the stem for a downward thrust. Using equipment as illustrated in Figure 1, the jar and its contents were placed on the scale platform and the movable dial adjusted to bring the "zero" reading under the indicator; with the curd knife in position directly over the coagulated milk, the air escape was then opened

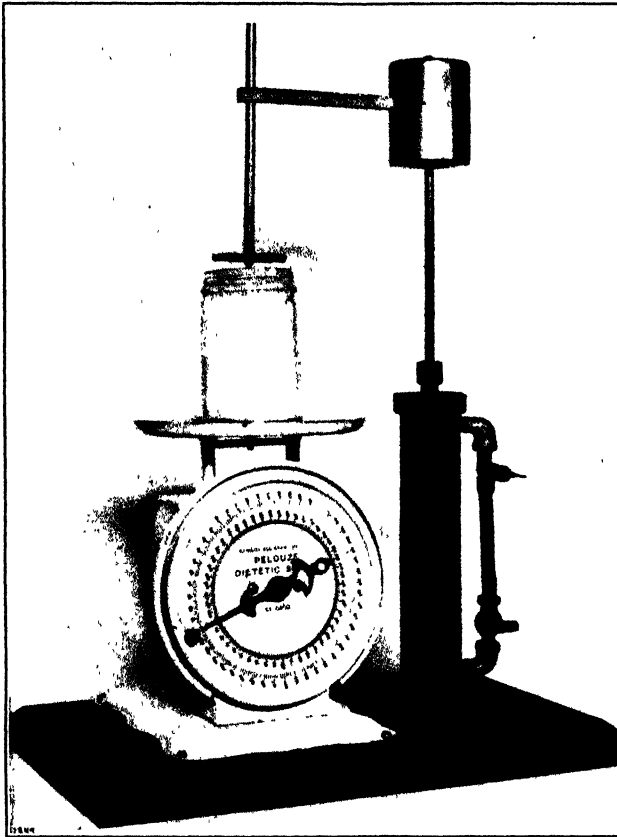


FIG. 1. EQUIPMENT USED FOR THE MODIFIED HILL CURD STRENGTH TEST.

to permit the curd knife to descend at a constant rate as regulated by a needle valve control on the air escape. The resistance encountered by the knife in passing through the curd could then be read off the dial directly.

The coagulation time with rennin was determined on 50 cc. of milk at 30° C. with 1 cc. of a 1 to 50 dilution of commercial rennet extract added. Wide mouth bottles with a capacity of 135 cc. were used (inside dimensions; body-diameter 4.8 cm., height 8 cm., neck-diameter 3 cm.). Equipment as illustrated in Figure 2 was used for tempering the samples, maintaining the temperature during the test and timing the coagulation. During the test the bottles rested on rollers at an angle of approximately 20 degrees from the horizontal and were immersed in the water up to the mouth. The rollers revolved to impart motion to the bottles at the rate of about 8 R.P.M. When the film of milk that clings to the inside surface of the inclined,

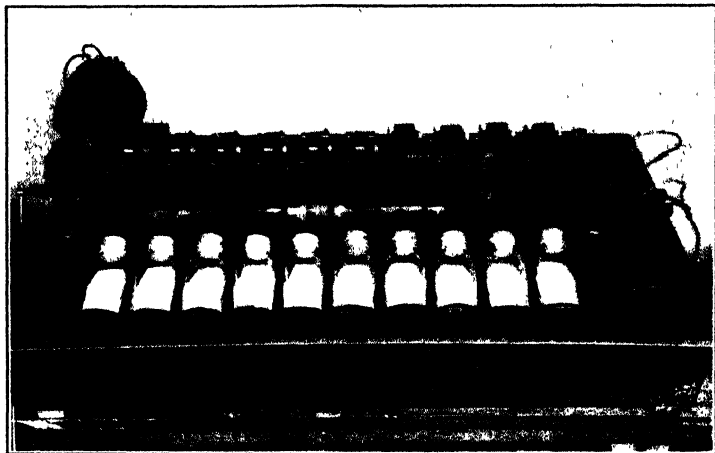


FIG. 2. EQUIPMENT USED FOR DETERMINING THE RENNET COAGULATION TIME OF MILK.

revolving bottle showed the first signs of graininess the test was terminated. With ten samples being timed simultaneously, the timing was accomplished conveniently by engaging and disengaging revolution counters at the start and end of the respective tests.

#### EXPERIMENTAL RESULTS

A number of curd strength tests were made on the milk from the individual cows in the normal and abnormal herds. Later the coagulation time with rennet was also determined. Table 1 gives a summary of the results. Samples on which both tests were run are averaged separately in Columns 5, 6 and 7, but are also included in the number of tests and the average curd strengths as shown in Columns 3 and 4. There is little evidence of correlation between the curd strength and rennet coagulation time.

The average curd tension of the milk from 31 normal cows (388 tests in all) was found to be 48.56 grams, while the average for 15 infected cows (191 samples in all) was 39.17 grams. The normal herd contained no cows giving milk with an average curd strength below 30 grams, while the infected herd contained 5 cows giving milk with a curd strength of less than 30 grams. The average rennet coagulation time for the normal herd was 7.56 minutes as compared with 10.61 minutes for the infected herd.

A number of the cows with infected udders were milked in such a manner as to keep the milk from the individual quarters separate. Pint samples from each quarter were brought to the laboratory for curd strength and rennet coagulation time measurements. Table 2 presents a summary of the results. Values given in bold face type represent infected quarters,

TABLE 1

*Curd strength and rennet coagulation time of milk from normal and infected udders*

COW	BREED	CURD STRENGTH		CURD STRENGTH & RENNET COAG.		
		No. of tests	Tension grams	No. of tests	Tension grams	Coag time minutes
(NORMAL UDDERS)						
Nell	Holstein	1	48.00	1	48.00	6.12
Girl	"	15	46.40	4	39.75	6.50
Homestead	"	16	36.94	4	36.25	12.43
Becky	"	6	52.17	2	49.00	8.20
Joan	"	15	59.73	4	59.25	6.85
Ida	"	16	63.88	4	63.00	6.38
Rose	"	16	69.00	4	61.00	4.94
Jo	"	16	50.38	5	50.00	13.97
Cordelia	"	13	59.31	2	45.50	14.22
Pearl	"	3	33.67	3	33.67	4.77
Lassie	Ayrshire	16	50.19	3	49.33	12.15
Quince	"	16	50.69	3	53.67	10.52
Priscilla	Guernsey	6	45.17	5	47.40	7.51
Faye	"	16	53.66	4	49.50	9.27
Mabel	"	9	37.22	3	28.33	4.83
Chum	"	9	31.56	1	29.00	6.42
Florence	Brown Swiss	7	35.28			
Fuschia	" "	6	37.33	3	32.33	5.59
Meridian	" "	8	35.50	4	32.75	7.63
Fairmaid	" "	14	53.50	3	57.00	5.69
Monona	" "	16	31.69	3	28.67	5.08
Fidelity	" "	15	34.47	3	25.67	6.44
Fan	" "	15	34.33	3	37.33	6.50
Eminent	Jersey	15	41.13	2	48.00	5.72
Logan	"	15	54.07	3	51.33	7.54
Safety Belle	"	11	58.18	3	58.67	5.89
Nita	"	17	72.12	5	68.00	6.42
Lotus	"	17	61.94	2	63.00	5.75
Lena	"	16	66.81	2	57.50	8.29
Nina	"	17	70.47	3	69.33	7.67
Fawn	"	10	30.70	3	32.00	7.59
Average		(388)	48.56	(94)	46.81	7.56
(INFECTED UDDERS)						
Alice	Holstein	15	27.47	5	30.20	22.36
Anita	"	14	52.78	4	47.50	8.90
Ruth	"	8	39.38	1	36.00	6.73
Inka	"	13	33.07	4	39.50	14.88
Champion	"	12	20.42	2	18.00	17.07
Pontiac	"	11	26.27	2	22.50	5.99
Allee	"	17	26.94	5	26.40	13.22
Lila	Ayrshire	7	38.29	4	36.75	7.63
N. Sally	Guernsey	17	48.59	5	47.00	7.98
C. Sally	"	13	38.00	1	32.00	4.71
Myrtle	"	14	54.86	3	56.33	10.64
Faith	Brown Swiss	15	26.07	4	25.75	13.38
Nanette	Jersey	17	73.53	5	70.40	6.48
Lee Murn	Brown Swiss	17	41.88	5	39.79	10.92
M-85	Holstein	1	40.00	1	40.00	8.26
Average		(191)	39.17	(51)	37.87	10.61

TABLE 2  
*Curd strength and rennet coagulation time of milk from individual quarters of infected udders*

DATE	COW	BREED	PH BY QUARTERS				CURD STRENGTH BY QUARTERS				COAG. TIME BY QUARTERS				AVE. CURD STRENGTH		AVE. COAG. TIME		
			RF	RR	LR	LF	RF	RR	LR	LF	RF	RR	LR	LF	Normal	Infected	Normal	Infected	
2-21	Ruth	Holstein	6.81*	6.63	6.51	6.50	23*	38	46	65	34.68*	9.05	5.63	5.38	49.7	23.0	6.69	34.68	
2-21	Inka	"	6.70	6.53	7.15	6.64	36	63	14	65	20.55	10.03	108.88	14.93	64.0	25.0	12.48	64.71	
2-21	Champion	"	6.78	6.82	6.79	6.65	29	22	22	40	21.90	28.38	21.04	9.91	34.5	22.0	15.40	24.71	
3-1	Pontiac	"	6.39	6.41	6.95	6.47	34	31	2	35	6.18	7.10	150++	6.24	33.3	2.0	6.51	150++	
3-1	Allee	"	6.49	6.65	6.49	6.42	27	12	27	33	13.70	39.14	16.39	11.07	29.0	12.0	13.72	39.14	
3-16	Allee	"	6.45	6.47	6.36	6.35	19	8	26	25	17.68	39.76	13.27	11.07	23.3	8.0	14.01	39.76	
2-20	Anita	"	6.83	6.63	6.61	6.80	45	42	41	11	9.30	7.59	7.16	44.53	41.5	28.0	7.37	26.91	
3-15	M-85	"	6.44	6.34	6.35	6.35	26	35	37	32	14.56	13.46	14.86	22.69	34.7	26.0	17.00	14.56	
2-19	Alice	"	6.79	6.74	6.73	6.76	24	27	29	33	37.07	24.90	26.79	23.92		28.2**		28.17**	
2-14	N. Sally	Guernsey	6.51	6.44	6.87	6.75	55	61	13	59	7.74	5.88	74.29	6.79	58.3	13.0	6.80	74.29	
3-16	N. Sally	"	6.40	6.33	6.84	6.39	42	57	18	54	10.03	5.99	91.75	6.55	51.0	18.0	7.52	91.75	
2-15	C. Sally	"	6.40	6.35	6.39	6.24	52	10	53	62	7.34	3.18	6.48	3.79	52.5	36.0	6.91	3.94	
2-20	Lee Murn	Brown Swiss	6.77	6.96	6.95	6.65	26	10	22	49	15.90	45.88	56.82	6.61	37.5	16.0	11.25	51.35	
2-14	Lila	Ayrshire	6.46	6.48	6.49	6.45	56	56	39	47	7.89	8.44	9.54	7.89	53.0	39.0	8.07	9.54	
3-16	Lila	"	6.39	6.40	6.47	6.41	55	52	37	50	8.56	8.56	10.46	8.07	52.3	37.0	8.40	10.46	
2-15	Nannette	Jersey	6.39	6.34	6.36	6.35	81	83	68	76	6.85	6.72	7.89	7.15	80.0	68.0	6.91	7.89	
3-9	61A	"	6.35	6.73	6.30	6.30	45	12	47	54	6.19	78.30	5.51	5.75	48.7	12.0	6.82	78.30	
3-7	3B	"	6.46	6.44	6.67	6.46	28	28	17	27	9.24	8.50	22.51	9.18	27.7	17.0	8.97	22.51	
Average												45.35	23.65			9.70	43.79		

\* Bold face figures represent infected quarters.

\*\* Not included in the average because there is no corresponding normal value.

the diagnosis having been made independent of this work as already explained. The average curd strength of the milk from normal quarters was 45.35 grams as compared with 23.65 grams for the milk from infected quarters. The rennet coagulation time was 9.70 minutes for the milk from normal quarters as compared with 43.79 minutes for the infected quarters. The most notable exception from these average indications is presented by the milk from C. Sally; one of the abnormal quarter samples showed a significantly higher curd strength than the normal quarters, and both abnormal quarters showed a decidedly more rapid coagulation with rennet. These results are partly explained by the pH as determined by the quinhydrone electrode. Evidently this cow had an infection which caused the milk to be more acid than normal. This was further confirmed by consulting the records of the veterinarian for January 9th (before this study was started) which showed the following pH values as determined colorimetrically: RF 6.8, RR 5.8, LR 6.7, LF 5.7.

Table 3 gives the results of a similar study of milk samples from individual quarters of cows which had been independently diagnosed as normal. While there are some variations in the curd strength and rennet coagulation of the milks from the four quarters of the same udder, in general these values were far more constant than those obtained with cows having one or more infected quarters as in Table 2. It is interesting to note that the average values obtained for normal cows when the milks were studied by individual quarters agreed quite closely with the average values obtained for normal cows (Table 1) when the entire milking was sampled.

#### CONCLUSION

Subclinical mastitis causes the milk to have a lower curd strength and to coagulate more slowly with rennet. This result was obtained both when entire milkings from normal and infected udders were compared as in Table 1, and when samples from infected quarters were compared with the milk from uninfected quarters of the same udder. This conclusion is further corroborated by the relatively greater constancy in the curd strength and rennet coagulation of milk from the four quarters of uninfected udders.

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# THE STANDARDIZATION OF THE BORDEN BODY FLOW METER FOR DETERMINING THE APPARENT VISCOSITY OF CREAM

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## INTRODUCTION

It is a well known fact, that, in spite of constancy of method of cream handling and processing, there is considerable daily and seasonal variation in its viscosity. Due to demand from production men of milk plants for a simple method of estimating cream body, a small simple efflux instrument was developed by Nair and Mook (1). A detailed description and illustrations of the Borden Body Flow Meter is given by them.

Since the Borden Body Flow Meter is coming into use in milk plants for determining the apparent viscosity of cream, it seemed desirable to compare the results obtained with it with results obtained by using a standard instrument such as the MacMichael viscometer. Although the MacMichael viscometer does not give true viscosity for mixtures of colloids and an emulsoid as found in milk and cream, it does give more accurate results than flow types of instruments with changing head pressure.

## EXPERIMENTAL

Solutions of sucrose ranging from 20 to 65 per cent were prepared with commercial cane sugar and distilled water. The percentage of sugar in the solutions was determined with saccharometers.

The viscosities of these sucrose solutions were determined in duplicate by the use of the MacMichael viscometer and the Borden Body Flow Meter. The operation of the MacMichael viscometer was in accordance with the recommendations in Eimer and Amend's Bulletin No. 280 using a number 30 wire calibrated with oils of known viscosity. The measurements with the MacMichael viscometer were taken after the oscillation of the bob became approximately constant. The operation of the Borden Body Flow Meter was in accordance with the recommendations of Nair and Mook (1). They also recommended the use of two interchangeable tips; one of 2.78 mm. diameter for cream containing 30 per cent of fat or more and one of approximately 1.97 mm. for thinner creams. Arbitrary temperatures for commercial purposes of 15.6° C. (60° F.) for heavy creams and 10° C. (50° F.) for light creams were chosen by them. The 2.78 mm. tip was used for all

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the work reported in this article. The viscosity determinations of the sucrose solutions were made at 20° C. (68° F.) and those of the cream were made at 15.6° C. (60° F.). The samples were tempered in a water bath in a room held at the temperature at which the determinations were made. Water in the bath of the MacMichael viscometer was also held at the temperature of the determination.

Viscosity determinations were made on two complete series of sucrose solutions. The results secured by the MacMichael viscometer and Borden Body Flow Meter are given in table 1 and the viscosities in centipoises as

TABLE 1  
*Viscosity of sucrose solutions as determined by the MacMichael viscometer and Borden Body Flow Meter at 20° C. (68° F.)*

SUCROSE SOLUTIONS	VISCOSITY WITH MACMICHAEL VISCOMETER	VISCOSITY WITH BORDEN FLOW METER
<i>per cent</i>	<i>centipoises</i>	<i>seconds</i>
First series of sucrose solutions		
21.1	3.0	28.0
39.5	7.0	40.0
W*	20.0	87.0
X*	31.0	119.5
61.4	73.5	279.0
63.5	109.0	412.0
64.4	128.8	492.6
Second series of sucrose solutions		
20.5	3.0	28.0
41.0	7.5	41.0
50.3	16.2	69.5
59.5	52.0	201.5
63.0	95.0	353.5
64.5	129.5	488.0
65.0	168.0	624.5

\* The percentage of sugar was not determined.

determined by the MacMichael viscometer are plotted against the seconds as determined by the Borden Body Flow Meter in Figure 1. Figure 1 gives a straight line relationship passing through zero which shows that the Borden Body Flow Meter gave correct results throughout the entire range for a true solution. The points indicating the seconds flow for the less viscous sucrose solutions are slightly below the line probably because the bore of the tip was too large to give accurate results with such solutions. The Borden Body Flow Meter, as previously mentioned, has a tip with a smaller bore to use with thinner liquids, but the results are only slightly in error with the large bore.

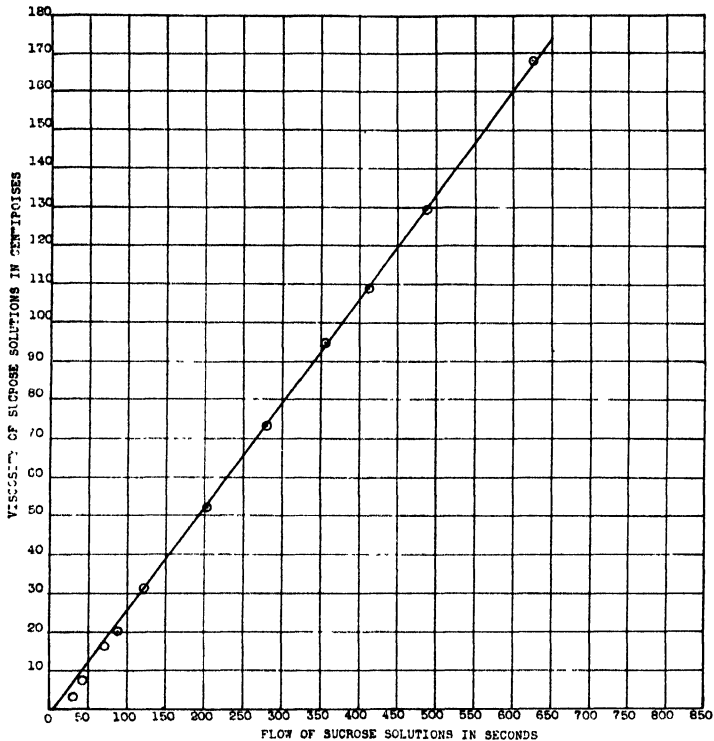


FIG. 1. The relationship of the apparent viscosity of sucrose solutions in centipoises and in seconds as determined by the MacMichael viscometer and Borden Body Flow Meter at 20° C. (68° F.).

The apparent viscosities of creams were determined with the MacMichael viscometer and Borden Body Flow Meter. The pasteurized milk from the station Jersey herd was separated at 61° C. (142° F.) and then the cream was heated to 74° C. (165° F.) and held for 10 minutes before cooling to 10° C. (50° F.) to reduce variations in viscosities due to fat clumping. This cream was standardized to the range of 30.0 to 52.5 per cent of fat, held for 20 hours at 4° C. (39.2° F.), then placed in  $\frac{1}{2}$ -pint bottles and tempered in a water bath to 15.6° C. (60° F.). All of the creams were held at this temperature during the time required for making the determinations. Tests were made at 2-hour intervals on duplicates of one of the samples to note any change in viscosity due to the holding period. The change in viscosity of the sample appeared to be within experimental error.

The averages of the results of 3 different lots of 30.0, 35.0, and 40.0 per cent cream, 2 different lots of 43.3, 45.0, 47.5, and 50.0 per cent cream, and from 1 lot of 42.0 and 52.5 per cent cream are given in Table 2. The

TABLE 2

*Viscosity of cream ranging from 30.0 to 52.5 per cent fat as determined by the MacMichael viscometer and Borden Body Flow Meter at 15.6° C. (60° F.)*

AVERAGED NO. TRIALS	CREAM  <i>per cent fat</i>	VISCOSITY WITH MAC MICHAEL VISCOMETER  <i>centipoises</i>	VISCOSITY WITH BORDEN FLOW METER	
			<i>Seconds</i>	<i>Centipoises*</i>
3	30.0	12.4	53.0	13.5
3	35.0	16.7	71.5	18.0
3	40.0	25.2	106.5	27.5
1	42.0	32.0	136.0	35.0
2	43.3	34.5	152.5	40.0
2	45.0	38.8	179.7	47.0
2	47.5	51.0	252.0	66.5
2	50.0	74.0	422.0	112.0
1	52.5	102.5	536.0	142.5

\* Seconds converted to centipoises by use of Fig. 1 based upon sucrose solutions.

viscosities in seconds of these creams obtained by the Borden Body Flow Meter were transposed to centipoises by means of Fig. 1 based upon standardization of the instrument with sucrose solutions. As can be seen from Table 2, the seconds of flow obtained by the Borden Body Flow Meter when converted to centipoises gave nearly the same results as the viscosity in centipoises as determined by the MacMichael viscometer for creams testing from 30.0 to 40 to 42 per cent fat. For creams of higher fat content there is a marked divergence in the viscosity in centipoises as determined by the Borden Body Flow Meter as compared with the MacMichael viscometer and this divergence increases with each succeeding increase in fat content. The Borden Flow Meter gave too high results which indicated that the results secured on sucrose solutions ought not be transposed to cream.

The relationship of the apparent viscosities of the cream in centipoises, omitting the 52 per cent cream, as determined by the MacMichael viscometer and the seconds as determined by the Borden Body Flow Meter are shown in Fig. 2. The results in seconds obtained with the Borden Body Flow Meter with cream show practically a straight line relationship with the results obtained with the MacMichael up to 25 centipoises, but thereafter the relationship is shown by a curved line.

A comparison of the two instruments was made on another series of creams ranging from 30 to 46 per cent milk fat which was separated from milk pasteurized in the usual way and not heated subsequently to a higher temperature. In this case some of the 35.0 and 40.5 per cent cream was treated by the method of Hening and Dahlberg (2) for the purpose of increasing its viscosity. The difference in viscosity due to this treatment was readily detected even at 15.6° C. (60° F.). Nair also reports from unpublished tests that differences in viscosity between heat-treated and non-

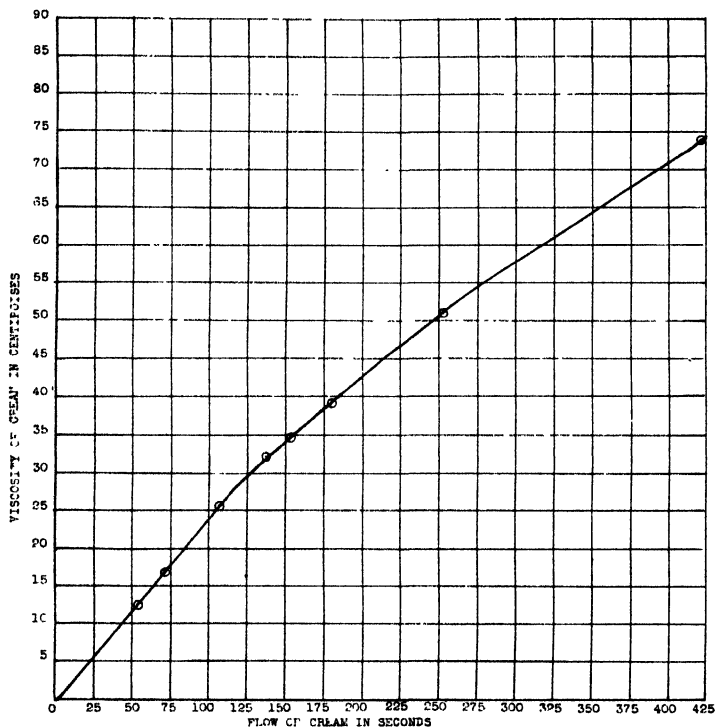


FIG. 2. The relationship of the apparent viscosity of creams in centipoises and in seconds as determined by the MacMichael viscometer and Borden Body Flow Meter at 15.6° C. (60° F.).

heat-treated creams are still demonstrable at a temperature of 15.6° C. (60° F.) using the flow meter. The results obtained with this series of cream were similar to those of the preceding series except that the viscosities of these creams were a little higher because the cream had not been heated to such a high temperature as in the previous tests. The results of these tests are given in Table 3.

As can be seen from Figures 1 and 2 and Tables 2 and 3, if the cream viscosity in seconds for 30, 35, and 40 per cent creams with the Borden Body Flow Meter was transposed to centipoises by means of Figure 1 based upon sucrose solutions, the reading would be 1 to 3 centipoises higher than that determined by the MacMichael viscometer. For creams of higher fat content from 42 to 50 per cent fat the difference would range from 3 to 38 centipoises too high. The difference in the determination with this instrument rapidly increases for creams having fat contents above 42 per cent. However, the flow in seconds of cream with the Borden Body Flow Meter can be transposed to centipoises by the use of Figure 2 based upon

TABLE 3

*Viscosity of cream ranging from 30 to 46 per cent fat as determined by the MacMichael viscometer and Borden Body Flow Meter at 15.6° C. (60° F.)*

CREAM	VISCOSITY WITH MAC MICHAEL VISCOMETER	VISCOSITY WITH BORDEN FLOW METER	
	centipoises	seconds	centipoises*
<i>per cent fat</i>			
30.0	13.0	52.0	13.5
35.0	18.0	74.5	19.0
35.0 treated	22.0	87.5	22.5
40.5	30.0	126.0	33.0
40.5 treated	46.5	215.0	56.8
46.0	60.5	303.0	80.5

\* Seconds converted to centipoises by the use of Fig. 1 based upon sucrose solutions.

results secured with cream. It should be possible by the use of Figure 2 based upon the standardization of the Flow Meter with cream to transpose the viscosity of cream in seconds to centipoises, the standard unit for expressing viscosity. In fact, if the seconds obtained with the Borden Body Flow Meter for the series of cream in Table 3 are converted to centipoises from Figure 2, the results check very close with those obtained with the MacMichael viscometer.

#### DISCUSSION AND CONCLUSIONS

In these experiments it has been shown that the Borden Body Flow Meter gave consistent results on the same and on different samples of cream. The accuracy of the apparatus was equally good on creams containing from 20 to 40 per cent of fat using only the large-bore tip of 2.78 mm. diameter. The same tip and temperature appeared satisfactory for the range of fat contents of market creams.

Standardization of the Borden Body Flow Meter was readily accomplished with sugar solutions and the seconds of flow showed a straight line relationship to the viscosity in centipoises. These results applied to the apparent viscosities of creams only up to 25 centipoises (about 100 seconds) which is the approximate viscosity of 35 to 40 per cent cream at 15.6° C. (60° F.). For creams of higher viscosity the Borden Body Flow Meter consistently gave results which were too high. However, results on very viscous creams may be transposed to approximate centipoises by the standardization data secured on cream, thereby making it possible for those using this Flow Meter to compare their results quite accurately with data reported in centipoises.

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# THE AGE THICKENING OF SWEETENED CONDENSED MILK

## I. SEASONAL VARIATIONS\*

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Manufacturers of sweetened condensed milk have frequently found that during storage their product would undergo a thickening to the extent that it no longer remained a syrupy liquid but had the consistency of a custard. In some cases the thickening may have been due to the growth of certain types of bacteria which represented a spoilage of the product, while in other cases the thickening was due to physical or chemical changes which took place on aging but which was not accompanied by any spoilage of the product. Bacterial thickening may be prevented by having the sucrose concentration high enough to inhibit the growth of bacteria. Colloidal or age thickening has been found to be most prevalent during the late spring and early summer. Even though age thickening is not accompanied by the spoilage of the product, such a heavy body is objectionable from the standpoint of the consumer. The factors involved in this type of thickening have not been well understood and manufacturers of this product have refrained from canning the milk for retail trade during the period of the year when the milk was unstable.

### REVIEW OF THE LITERATURE

*Effect of Storage Temperature:*—Rogers, Deysher and Evans (1) found that sweetened condensed milk thickened more rapidly at high storage temperatures, but that the increase in viscosity was not in direct relation to the temperature. They reported no increase in viscosity when the milk was stored at 10° C. (50° F.) for 121 days, a slight increase at 20° C. (68° F.), and a marked increase in viscosity at 30° C. (86° F.) and higher.

Miyawaki (2) states, "In general, it may be said that about three weeks of incubation corresponds to about six months of aging under natural conditions." However, he does not state the temperature of incubation.

Leighton and Mudge (3) determined the length of time for thickening to begin in condensed milk stored at various temperatures and concluded that the reaction at the higher and lower temperatures was identical. These investigators have also shown that the heat coagulation of the casein of milk is an endothermic reaction and that the heat absorption comes from the precipitation of calcium and magnesium as phosphates and citrates. The

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heat absorption obtained with condensed milk was greater than that obtained from a serum made up according to the formula of Van Slyke and Bosworth. If enough extra calcium was added to make up for the amount combined with the protein the heat absorption of the serum was commensurate with that obtained with condensed milk.

*Effect of Forewarming Temperature:*—Hunziker (4) found that forewarming temperatures up to 167° F. had a slight tendency toward stabilizing the milk over the unforewarmed milk. From 167° F. to boiling the effect was to unstabilize the milk, while temperatures above boiling increased the stability and diminished the tendency to thicken. He recommends that the forewarming temperature be limited to 167–170° F. for 10 minutes in order to control the thickening.

Leighton and Deysher (5) found that a forewarming temperature of 65° C. (149° F.) slightly stabilized the product as compared to that made from unforewarmed milk; at 75° C. (167° F.) there was also a slight stabilizing effect; at 85° C. (185° F.) a decrease in stability set in, which was very marked at 95° C. (203° F.); and increased stability followed upon forewarming at 110 and 120° C. (231 and 248° F.).

Rogers, Deysher and Evans (1) have shown that milk forewarmed at 80° C. (176° F.) thickened much more rapidly than milk forewarmed at 60° C. (140° F.). They reported that satisfactory results may be obtained by forewarming to about 63° C. (145.4° F.) for 20 to 30 minutes. This temperature reduced the tendency of the milk to thicken during storage but there was danger of fat separation.

Sommer (6) reported that high forewarming temperatures in the case of evaporated milk tended to stabilize the product when subsequently subjected to sterilizing temperatures. Leighton and Deysher (5) and Leighton and Mudge (3) have shown that when stability was plotted against forewarming temperature the curve obtained for sweetened condensed milk was the reverse of the curve obtained for evaporated milk.

According to Leighton and Mudge (3), continued forewarming at 95° C. resulted in a stabilization of the product, but the effect was not as marked as in the highly forewarmed product. However, their stability figures were not consistent with their observations on the viscosity.

Leighton and Deysher (5) concluded that the temperature of forewarming was the major factor in determining the resistance to heat of both evaporated and condensed milk and that the time of forewarming was not so important but could not be neglected.

*Effect of Air:*—Experiments by Rogers, Deysher and Evans (1) in which samples of sweetened condensed milk were stored in evacuated bulbs, showed that air was not a factor in the age thickening of the milk.

*Effect of Homogenization:*—Rogers, Deysher and Evans (1) showed that while homogenization of sweetened condensed milk had the effect of increas-

ing the initial viscosity it was without appreciable effect on the progressive increase in viscosity.

*Effect of Reaction:*—Rogers, Deysher and Evans (1) reported that variations in the acidity within reasonable limits had no appreciable effect on the viscosity of sweetened condensed milk. Batches of milk in which they adjusted the acidity before condensing from pH 6.51 to 6.30, 6.41 and 6.63 showed no real difference in viscosity upon aging. However, in one experiment in which the reaction had been materially changed the milk thickened in the pan. In the above experiments the skim milk was forewarmed at 63° C. (145.4° F.) and the samples showed practically no increase in viscosity at the end of 30 days' storage at 30° C. (86° F.). Hunziker (4) is of the opinion that had they used a higher forewarming temperature the samples with the higher acidities would have probably shown a decrease in stability toward age thickening.

Leighton and Mudge (3) found that the pH of the artificial serum of Van Slyke and Bosworth changed from pH 6.59 to approximately 6.25 upon precipitation produced by heating, but there was no change in the reaction of sweetened condensed milk upon thickening.

*Effect of the Sucrose:*—Rogers, Deysher and Evans (1) found that the cane sugar concentration had only a minor influence on the initial viscosity and probably none at all on the progressive increase in viscosity. In these experiments on the effect of sugar concentration a forewarming temperature of only 48° C. (118.4° F.) was used and Hunziker (4) points out that had they used a forewarming temperature high enough to encourage colloidal thickening that probably the sucrose concentration would have had an influence on the subsequent thickening.

Hunziker (4) has found that increasing the amount of sugar tended to minimize the thickening tendency, even in the milk made in early summer. He suggests that probably the higher concentration of sucrose may physically hinder hydration and swelling.

Leighton and Deysher (5) found that sugar exerted its influence on the stability in the forewarming process, in the evaporating process and in the coagulating process. Its effect was to unstabilize the 95° C. forewarmed product and to stabilize the 120° C. material. Based upon the findings of Leighton and Mudge (3) that the heat absorption incident to coagulation comes from the precipitation of calcium and magnesium, Leighton and Deysher (5) believe that the sugar effect has its action upon the metallic salts of milk since cane sugar can react with calcium and since some calcium and magnesium salts dissolve in sugar solutions.

Leighton and Mudge (3) are of the opinion that the sugar accelerates the removal of the calcium from an active state under the conditions of prolonged forewarming.

Ramsey, Tracy and Ruehe (7) found that where dextrose was used to

replace part of the sucrose in sweetened condensed milk that the physical thickening was greatly accelerated, but which could be prevented by adding the corn sugar as a concentrated solution near the end of the run instead of adding it to the milk before forewarming.

*Effect of Fat:*—Miyawaki (8) has shown that condensed milk made from fat-poor milk does not in general remain syrupy long, but tends to become pasty. He recommends that the raw milk should contain at least 3 per cent fat.

*Effect of Casein and Albumin:*—Rogers, Deysher and Evans (1) found that the casein and albumin were the milk constituents directly responsible for age thickening, and that casein had, by far, the greatest influence. Albumin became a factor in the thickening only when forewarming temperatures above its coagulation point were used.

*Effect of Concentration of Milk Solids:*—Rogers, Deysher and Evans (1) have shown that increasing the concentration of the milk solids increased the tendency of the sweetened condensed milk to thicken. The tendency to thicken was not in direct proportion to the concentration of the solids but increased more rapidly at a concentration of 26 to 28 per cent than at lower concentrations.

*Effect of Salts:*—Rogers, Deysher and Evans (1) found no appreciable effect on the viscosity by adding 2.8 grams of calcium citrate to 3,500 grams of skim milk when the finished product was held at 30° C. (86° F.) for 58 days. With the addition of enough monobasic phosphate to increase the total phosphates of the milk 5 per cent there was no significant difference in the viscosity although it was somewhat more viscous than the control batch. The addition of phosphates in amounts to raise the total phosphates by 7 to 11 per cent caused a decided increase in the thickening tendency in 23 days, and much more so in 44 days. These investigators also found that the addition of copper salts failed to show any increase in viscosity over the control.

*Effect of Vacuum Pan Temperature:*—Hunziker (4) found that finishing the batch at a relatively high temperature (150° F. or above) yielded a product with higher initial viscosity and thickening tendency than finishing at relatively low temperatures (120 to 130° F.).

Miyawaki (2) recommends keeping a vacuum above 26 inches on the pan in order to secure a good keeping quality milk.

*Effect of Season of the Year:*—As is well known by manufacturers of sweetened condensed milk, and as pointed out by Hunziker (2), the greatest difficulty with rapid thickening occurs during the late spring and early summer.

Sommer (6) has pointed out that the troublesome heat coagulation in the case of evaporated milk is most prevalent during the fall, winter, and early spring.

## EXPERIMENTAL METHODS

*The Vacuum Pan:*—The various batches of sweetened condensed milk used in these experiments were made in an experimental pan devised to approximate commercial conditions as nearly as possible and at the same time permit accurate control as well as the use of a small amount of milk for a trial. The pan is shown in figure 1. Condensing was done in balloon

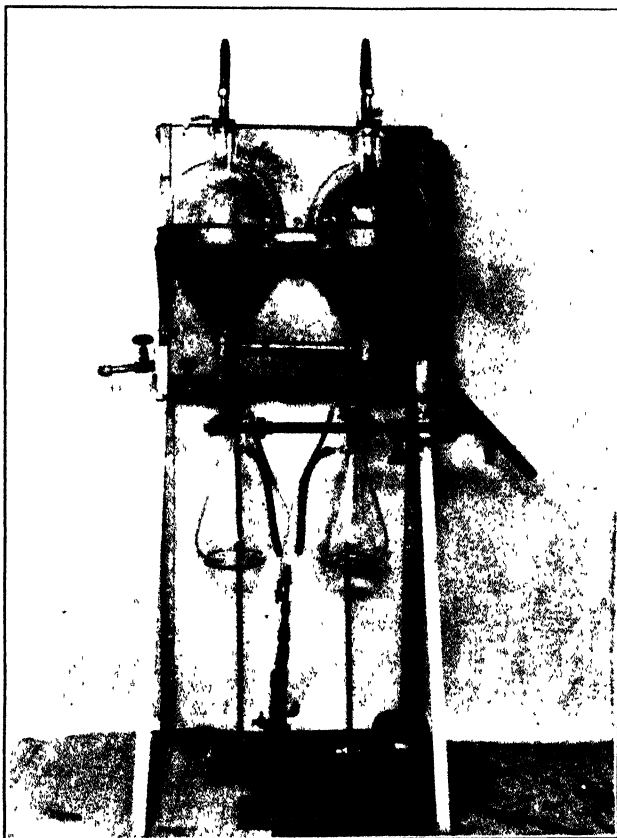


FIG. 1. Experimental vacuum pan

flasks of 12 liters capacity, the heat being applied by means of live steam in a copper-jacket surrounding the lower one-third of the flask. The vacuum was maintained with a motor-driven vacuum pump, attached, through a suction flask, to a copper coil condenser submerged in a tank of running water. The condenser was attached to the condensing flask with

a rubber stopper which also served to hold a thermometer and a glass stop-cock used to regulate the flow of milk into the flask. Two such units were made into one piece of apparatus so that a control batch could be run at the same time as a variable factor was being tried on the other batch. The apparatus was also provided with a vacuum gauge. With this arrangement it was possible to control accurately the temperature and vacuum in both flasks.

*Milk*.—Except in the experiments with the milk of individual cows, mixed milk from the University Creamery was used. About 8 pounds of milk were required for a batch and were standardized to give 3.4 pounds of the finished product containing approximately 8 per cent fat, 20 per cent milk solids not fat, and 44.1 per cent sucrose.

*Forewarming*.—The forewarming was done in five-gallon “shot gun” cans placed in a water bath heated with steam. In some cases where a high forewarming temperature was desired the steam was introduced directly into the milk. Unless otherwise stated, the sugar was added to the milk before heating was commenced and the milk was forewarmed to 88° C. (190.4° F.) for a period of 10 minutes before drawing it into the pan.

*Condensing*.—The vacuum was regulated so that condensing was done at a temperature of 55° C. (131° F.). In finishing the batch the vacuum was increased, thus decreasing the temperature of the milk so as to avoid any possibility of superheating. It required about 2½ hours to complete the evaporation. After the condensing was judged complete the flasks were disconnected and weighed on a sales counter scale and distilled water added to make up the contents to the desired weight. After a little experience it was possible to judge the end point so that it was seldom necessary to add more than a small amount of water.

*Storage of the Condensed Milk*.—In some of the first experiments the milk was stored in tin cans and in one-half pint milk bottles sealed with paraffin. In the later trials, when a different method of measuring the viscosity was adopted, the milk was put up in rubber-stoppered 50 × 400 mm. test tubes. Storage was made at 37° C. (98.6° F.) in order to hasten thickening.

*Viscosity Measurements*.—In the early trials the viscosity of the condensed milk was measured with the MacMichael viscometer using a number 30 wire, but for various reasons this instrument was not suited to this work. The use of this instrument necessitated the removal of the milk from the container and stirring. Stirring the sample decreased the viscosity which made it difficult to stir all samples alike. An attempt was made to obtain the “basic” viscosity by first forcing the milk through a capillary tube, but this was found to be impractical because the milk began to thicken again as soon as it left the tube. However, what was really desired was not the “basic” viscosity of the milk but its consistency as the consumer would find it upon opening the container.

These objections to the use of the MacMichael viscometer were eliminated by the falling-sphere method of measuring viscosity, using a vacuum tube arrangement to detect when a steel ball passed through two coils of wire placed 22.7 cm. apart. When a viscosity measurement was to be made the tube of milk was inserted through the coils, a steel ball dropped in the top, and its fall timed with a stop-watch while it fell the 22.7 cm. The apparatus was designed to show a deflection on a galvanometer needle when a steel ball passed through the coils, causing a change in the amplitude of oscillations in the oscillating circuit. After making a measurement the steel ball was allowed to remain in the tube until the milk was discarded. This method was very rapid and had the advantage of permitting a viscosity measurement to be made with the minimum of disturbance to the milk. A diagram of the apparatus, including a list of the various parts is shown in figure 2.

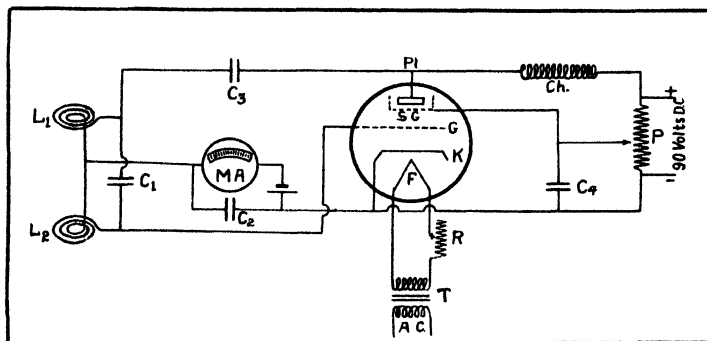


Fig. 2. Diagram of Vacuum Tube Viscometer

$L_1, L_2$ —10 turns each of No. 16 or No. 18 double cotton covered enameled wire.

Ch.—1000 turns of No. 38 enameled wire or R.F. choke for 3000 kilocycles.

$C_2$ —0.0005 microfarad mica condenser. Select one having less than 0.01 milli-ampere leakage.

$C_3, C_4$ —0.5 microfarad paper condensers.

$C_5$ —0.01 microfarad paper condenser.

M.A.—0 to 1.0 milliamper meter.

P—10,000 ohm, wire wound only, potentiometer of good quality.

R—2.0 ohm rheostat, wire wound only.

T—110 volt A.C. transformer with 2.5 volts output.

Screen grid vacuum tube, type 235.

Pl.—Plate.

S.G.—Screen grid.

G.—Grid.

K.—Cathode.

F.—Filament.

Viscosity measurements were made at 37° C. (98.6° F.), the temperature of storage, and the results calculated to absolute units according to Stokes'

formula. Steel balls of three different diameters were used, namely: 0.6345, 0.9520 and 1.2695 centimeters. Where the viscosity of the samples was so great that it required more than 2 minutes for a steel ball with a diameter of 1.2695 cm. to fall 22.7 cm. through the milk it was reported as being too viscous to measure. When the viscosity was high there was a tendency for the steel ball to slide to the side of the tube rather than to fall straight down.

### 1. SEASONAL VARIATIONS

The prevention of fat separation in sweetened condensed milk depends upon having the viscosity of the milk great enough to prevent the rise of the fat globules. Normal sweetened condensed milk has a viscosity of about 6 to 8 poises at 37° C. (98.6° F.) shortly after it is dropped from the pan, but a further increase in viscosity during storage is desirable because this initial viscosity is not great enough to prevent fat separation, especially if the milk is held in storage for any great length of time.

Normal sweetened condensed milk will show a gradual increase in viscosity during storage, the rapidity of the thickening depending upon the temperature at which it is held. However, there is a certain period during the year when the milk has the tendency to thicken abnormally fast, especially at room temperature or higher. During this unstable period it is not uncommon to have a sample thicken so rapidly that at the end of 24 hours' storage at 37° C. (98.6° F.) the container may be inverted without loosing its contents.

This unstable period is usually referred to as occurring during the late spring and early summer, especially during May and June. The cause of the instability during this season of the year is not definitely known, but is no doubt due to some change in the composition of the milk at this time. This being the case we should expect the date of change from stable to unstable milk to vary with the community and from year to year.

The milk used in these trials was taken from the raw milk storage tank of the University Creamery and consisted of the mixed milk from several farms. This milk was fairly representative of the milk from the area of Madison, Wisconsin.

In the spring of 1932 the batch made on April 23 thickened in a normal manner during storage, while that made on April 24 and the subsequent batches thickened abnormally fast. After May 20 no condensing was done until June 24 when the milk was relatively more stable than it was during May and the latter part of April. These results are shown in Table 1.

In 1933 condensing was not begun until April 15 and the milk was already unstable at that date as shown in Table 2. The last batch was condensed on May 27 and the milk was still unstable.

Table 3 shows the progressive increase in viscosity during storage of six batches of sweetened condensed milk made during a period extending

TABLE 1  
*Seasonal changes in stability during the spring and summer of 1932*

DAYS AGING	APRIL 18	APRIL 23	APRIL 24	MAY 2	MAY 9	MAY 14	MAY 20	JUNE 24	JULY 7	JULY 19	JULY 20
0	4.9	6.2	7.4	6.2	4.9	24.3	52.9	7.4	8.7	6.5	7.4
1			230.0				*	112.2	36.2	14.8	18.6
2		44.6		138.5	130.0	*					
3				*							
4	162.3	163.2		422.0	1190.0				273.1		
5								792.2			
6	317.8			1981.0	*				740.0	69.3	568.0
8											
9	293.0	460.0						568.0			
10				2031.0							
11											
12											
13		258.1									
14	374.2			*					773.0	28.4	660.0
16		213.0						525.7			
18	323.0							1088.0			
20											
21	377.6	407.6									
25											
26											
27								795.0			
30	992.0	505.1							1835.0	1116.0	

\* Samples too viscous to measure.

Storage temperature 37° C. (98.6° F.).

Forewarming temperature 88° C. (190° F.) for 10 minutes.

TABLE 2  
Seasonal change in stability during spring 1933

DAYS	APRIL 15	APRIL 22	APRIL 29	MAY 4	MAY 15	MAY 25	MAY 27
	Viscosity in Poises						
0	16.1	41.0	14.8	18.6	22.3	38.4	44.6
1	144.0	1050.0		584.0		*	
2	865.0	*		2205.0			1850.0
3	*		*	*			
5					*		*

Forewarming temperature 88° C. (180° F.) for 10 minutes

Storage temperature 37° C. (98.6° F.).

\* Too viscous to measure.

from April 27, 1934, to May 29, 1934. The batch made on May 15, 1934, had a normal initial viscosity and thickened in a normal manner, while the batch made on May 19, 1934 had a high initial viscosity and thickened

TABLE 3  
Seasonal change in stability during spring 1934

DATE OF CONDENSING	APRIL 27	MAY 8	MAY 15	MAY 19	MAY 24	MAY 29
Sucrose	44.1	44.1	44.1	44.1	44.1	44.1%
Fat	7.55	8.29	7.52	8.31	8.05	7.16%
Milk solids not fat	20.67	20.73	20.83	21.43	20.80	20.34%
Days	Viscosity in Poises					
0	7.4	7.4	6.2	31.0	17.3	38.4
1		25.6	14.8		1038.0	1488.0
2				*		*
3	100.4	125.0				
4			58.5	Solid	*	
5	131.3					
7		135.0				
8			82.4			
10	529.0					
11		158.9				
13			95.0			
14	470.7					
17		204.1				
18	539.8					
20		178.7	124.1			
22	740.1					

Forewarming temperature

Storage temperature 85° C (185° F) for 10 minutes. 37° C (98.6° F).

\* Too viscous to measure.

abnormally fast. In 1934 the unstable period did not commence until approximately a month later than in 1933 and 1932.

Trials could not be carried out during the summer of 1933 and 1934, so it is not known when the unstable period ended. The data given in Table 1 tend to show that the change again from unstable milk is not as abrupt as is the change from stable to unstable milk but occurs during the latter part of June and the first part of July.

That the change from stable to unstable milk is very abrupt is shown in Table 1 where the milk condensed on April 23, 1932, was stable while that condensed on April 24 was very unstable. Table 3 shows that during the spring of 1934 the change occurred between May 15 and May 19. If batches had been condensed during the intervening 3 days it might have been found that the change occurred over night.

#### DISCUSSION

It is impossible to state any definite limit of viscosity above which the milk may be considered as unstable and below which the milk may be said to be stable. However, numerous trials have shown that when unstable milk was stored at 37° C. (98.6° F.) the thickening was usually so rapid that it attained a viscosity of over 3000 poises (too viscous to measure) within a few days after manufacture, while the viscosity of stable milk under the same conditions of storage was usually below 1000 poises at the end of 3 weeks. The initial viscosity of stable sweetened condensed milk averaged from 6 to 8 poises, while unstable milk was usually somewhat higher. However, it was not always possible to predict the rate of thickening of the milk from the initial viscosity.

Hunziker (4) suggests that probably the cause of the unstable period during the spring is due to a large majority of the cows freshening at that time thus causing a high albumin content in the milk. This explanation would not hold for this territory where most of the cows freshen earlier in the year and at best would not explain the abrupt change to unstable milk. The milk at this season of the year is no doubt relatively high in albumin and it may be a factor.

It has been observed in the evaporated milk industry that the change from unstable to stable milk in the spring also occurs very abruptly but this has been explained as being due to the increased citrate content in the milk caused by green pasture feed. Unstable sweetened condensed milk cannot be attributed entirely to the change in the milk occasioned by green feed, but it may be a factor. The change to green feed would not explain the fact that the unstable period in 1934 did not occur until approximately a month later than in the two previous years. The spring of 1934 was relatively hot and dry. During the latter part of June and July in 1932 the milk was much more stable than it was during May even

though the cows were still getting plenty of good green feed. In some trials where the milk of individual cows was condensed it was found that some cows (especially Jerseys) gave milk that was consistently unstable even though they were on winter rations.

#### CONCLUSIONS

The unstable period for sweetened condensed milk begins very abruptly and may occur any time from the middle of April to the middle of May in the territory of Madison, Wisconsin.

The change back from unstable to stable milk appears to occur more slowly and takes place during the latter part of June and July.

The freshening of the cows or the time at which they are turned out on grass cannot be correlated directly with the period during which sweetened condensed milk is unstable toward age thickening.

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# THE DIKETONE PRODUCED WHEN BUTTER CULTURES ARE STEAM DISTILLED WITH FERRIC CHLORIDE ADDED\*

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The relationship of diacetyl to the desirable flavor of butter has been established by the work of various investigators. When a satisfactory butter culture is steam distilled and the distillate treated with suitable reagents, a brick red precipitate is formed that is commonly considered to be nickel dimethylglyoximate, a derivative of diacetyl. Much more precipitate is formed if ferric chloride is added to the butter culture before distillation, and this is interpreted as indicating the formation of relatively large amounts of diacetyl through an oxidation of acetylmethylcarbinol present in the culture.

Since certain homologues of diacetyl have been assumed to be valuable for the purpose of giving oleomargarine a desirable flavor and aroma (3) and also because these homologues are similar to diacetyl from various standpoints, it seemed advisable to determine whether or not the diketone obtained on distilling a butter culture with ferric chloride is diacetyl. The general procedure followed was to prepare the usual nickel salts from the distillates of butter cultures and then determine the nickel contents for comparison with the theoretical nickel content of nickel dimethylglyoximate.

## METHODS

A relatively large amount of each butter culture was steam distilled, after adding ferric chloride, and the distillate treated with hydroxylamine hydrochloride, sodium acetate, and nickel chloride solutions; 200 ml. of 40 per cent ferric chloride solution (40 gm. made up to 100 ml. with distilled water) were used per liter of butter culture, and the other reagents were employed in amounts sufficient to insure complete precipitation of the diketone present. Each precipitate was washed repeatedly with distilled water by decantation and then collected, with suction, on a crucible having a fused-in sintered filter disc; the precipitate was also thoroughly washed on the crucible. The salt was removed from the crucible and dried at slightly more than 100° C. During the decantation there was some loss of the precipitate, so the salt was determined quantitatively on a 200-gm. portion of each butter culture, using the procedure followed by Michaelian

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and Hammer (2) for determining both the diketone present originally and that resulting from an oxidation with ferric chloride.

The nickel content of each salt was determined by decomposing, with sulfuric and nitric acids, a portion that had been thoroughly dried and precipitating the nickel as dimethylglyoximate, which was collected and weighed.

#### RESULTS OBTAINED

The results obtained are given in Table 1. The percentages of nickel in the various salts prepared from butter cultures ranged from 20.14 to 20.30, all of the values being close to the theoretical for nickel dimethyl-

TABLE 1

*Nickel content of salts obtained when butter cultures were steam distilled with ferric chloride and the distillates treated with hydroxylamine hydrochloride, sodium acetate and nickel chloride*

*Butter cultures developed at 21° C.*

BUTTER CULTURE			GM. OF PURIFIED NI SALT OBTAINED	% NI IN NI SALT	MG. NI SALT OBTAINED QUANTITATIVELY FROM 200 GM. CULTURE
No.	General quality	Ml. distilled			
15	excellent	3,000	0.5558	20.24 20.20 } 20.22	45.8
146	fair	2,750	0.3524	20.29 20.31 } 20.30	33.2
15	good	8,500	1.1838	20.25 20.20 } 20.225	39.6
232	good	10,000	1.7534	20.17 20.11 } 20.14	43.5
15*	**	10,000	3.2555	20.22 20.25 } 20.235	84.1

\* 0.15% citric acid crystals added to milk from which butter culture was made.

\*\* Flavor and aroma were typical of a butter culture made from milk to which citric acid had been added.

glyoximate<sup>1</sup> and distinctly higher than the theoretical values for the higher homologues. An average nickel content of 20.30 per cent was obtained on the nickel salt made from a commercial preparation of diacetyl. When citric acid was added to the milk used in preparing the butter culture, the salt obtained had the same nickel content as the salts obtained when no citric acid was added to the milk. The amounts of nickel salt obtained quantitatively per 200 gm. of the various cultures were normal for butter cultures of a satisfactory quality (1).

<sup>1</sup> The theoretical Ni content of nickel dimethylglyoximate = 20.32 per cent; of nickel ethylmethylglyoximate = 18.52 per cent; of nickel diethylglyoximate = 17.02 per cent.

From the data presented it appears that the diketone steam distilled from butter cultures after the addition of ferric chloride is diacetyl rather than one of the homologues and that if homologues are present they are limited to relatively non-significant amounts.

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## MOLDINESS IN ROMANO CHEESE\*

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### INTRODUCTION

A factory manufacturing Cacio Pecorino Romano cheese, more commonly known as Romano cheese, from cows' milk reported that they were having considerable trouble with mold developing in the center of many of the cheeses. Inasmuch as this was undesirable in this type of cheese, they were anxious to find the cause and remedy the condition. Several cheeses in which the mold was present in the center and several cheeses in which no mold was present were submitted for study and analysis.

In making Romano cheese the procedure usually followed is to soak the cheese in a 30° salometer brine for about 3 days. After this the cheese is removed from the brine and salt is rubbed into it every day for four days. The cheese maker at this particular factory had punched the cheese full of holes to allow greater penetration of the brine. After the cheese had been removed from the brine and stored for aging, the holes permitted the air as well as mold spores to enter the cheese. In all cases the moisture content was sufficient to permit the growth of mold. Doane and Lawson<sup>1</sup> and also Matheson<sup>2</sup> in the revised edition of U. S. D. A. Bulletin 608, p. 36, in describing Romano cheese and its manufacture, referring to salting, state: "This process is sometimes facilitated by punching several holes in the cheese." In the light of the present work, it is inadvisable to follow this procedure.

### METHOD OF ANALYSIS

Samples of the moldy cheese were ground in a sterile mortar with sterile sand and plated on malt extract agar. A pure culture of mold was isolated and identified as *Penicillium italicum*.

In order to study the conditions which favored the development of this mold in the cheese, analyses were made of the moisture and sodium chloride content of cheese that had molded and of cheese that had not molded. The moisture content was determined by weighing approximately 10 grams of the cheese in a platinum crucible and drying in a vacuum oven at 56° C.

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<sup>1</sup> Doane, C. F., and Lawson, H. W. Varieties of cheese: descriptions and analyses. U. S. D. A., Bul. 608, (1918).

<sup>2</sup> Doane, C. F., Lawson, H. W., and Matheson, K. J. Varieties of cheese: descriptions and analyses. U. S. D. A., Bul. 608, (1928).

TABLE 1  
*Diagram of section of cheese in which no mold was present showing the percentage of moisture  
 and sodium chloride present*

1 H <sub>2</sub> O = 12.0 NaCl = 7.4	2	3	4	5	6	7 H <sub>2</sub> O = 13.0 NaCl = 7.5
8	9 H <sub>2</sub> O = 15.0 NaCl = 7.7	10 H <sub>2</sub> O = 18.0 NaCl = 7.6	11 H <sub>2</sub> O = 21.0 NaCl = 7.5	12 H <sub>2</sub> O = 15.0 NaCl = 7.8	13 H <sub>2</sub> O = 16.0 NaCl = 7.9	14
15	16 H <sub>2</sub> O = 21.0 NaCl = 7.8	17 H <sub>2</sub> O = 15.0 NaCl = 8.0	18 H <sub>2</sub> O = 17.0 NaCl = 7.8	19 H <sub>2</sub> O = 16.0 NaCl = 7.8	20 H <sub>2</sub> O = 17.0 NaCl = 7.6	21
22 H <sub>2</sub> O = 17.0 NaCl = 7.3	23	24	25 H <sub>2</sub> O = 16.0 NaCl = 7.8	26	27	28 H <sub>2</sub> O = 20.0 NaCl = 7.5

until the weight was constant. The sodium chloride content was determined by igniting one gram of cheese in a platinum crucible. The resulting ash was then dissolved in 10 cc. of water and titrated with  $n/10$   $\text{AgNO}_3$ , using a one per cent  $\text{K}_2\text{CrO}_7$  solution as the indicator.

In order to study the relationship between the moisture and sodium chloride content and moldiness in the various samples of cheeses, the cheese was cut into slices about one inch thick and these were diced and numbered. The results of typical analyses are given in the following diagrams representing the slices of the diced cheese.

TABLE 2  
*Diagram of section of moldy cheese showing relation between the percentage of moisture and sodium chloride and the presence of mold in the cheese. Mold confined to section 8*

1 $\text{H}_2\text{O} = 15.7$ $\text{NaCl} = 7.5$	2 $\text{H}_2\text{O} = 18.4$ $\text{NaCl} = 7.5$	3 $\text{H}_2\text{O} = 18.0$ $\text{NaCl} = 7.0$	4 $\text{H}_2\text{O} = 19.9$ $\text{NaCl} = 6.7$	5 $\text{H}_2\text{O} = 11.1$ $\text{NaCl} = 7.5$
6	7	8 $\text{H}_2\text{O} = 24.5$ $\text{NaCl} = 6.0$	9	10
11	12 $\text{H}_2\text{O} = 20.1$ $\text{NaCl} = 5.8$	13 $\text{H}_2\text{O} = 19.8$ $\text{NaCl} = 7.2$	14 $\text{H}_2\text{O} = 23.9$ $\text{NaCl} = 6.3$	15
16 $\text{H}_2\text{O} = 17.7$ $\text{NaCl} = 7.4$	17	18 $\text{H}_2\text{O} = 18.6$ $\text{NaCl} = 7.5$	19	20 $\text{H}_2\text{O} = 16.1$ $\text{NaCl} = 8.2$

#### INOCULATING CHEESE WITH MOLD

Squares of cheese from a cheese which showed no mold were placed in deep culture dishes. Mold spores from the mold isolated from the moldy cheese were then spread on the surface of the cheese and incubated a week with no growth resulting. One cc. of water was then added to the squares, but no growth resulted. It was necessary to repeat this twice before the mold grew on the surface of the cheese.

Our results would indicate that the moisture content of the cheese would have to be approximately 25 per cent and the salt content not greater than 6.0 per cent to permit mold growth to take place. Other sections of the cheese having a greater salt content or a smaller amount of moisture were not affected. In the cheese where no mold growth occurred, the moisture content was less than 25 per cent and the salt content greater than 6 per cent.

These results most certainly indicate that in making Romano cheese, it is not advisable to punch holes in the cheese to facilitate the penetration of the brine into the interior. This may permit molds to grow in the center. Molds developed in every cheese in this factory so punctured.



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## THE INFLUENCE OF STREPTOCOCCIC INFECTION OF THE UDDER ON THE FLAVOR, CHLORIDE CONTENT, AND BAC- TERIOLOGICAL QUALITY OF THE MILK PRODUCED\*

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Much attention is being given today to the factors affecting the quality of dairy products. It is recognized that the quality of the raw or basic material plays an important rôle in the general quality of the processed product. For this reason as well as for the assurance of safety from the public health standpoint is the milk supply subjected to inspection.

At the receiving platform, the milk is accepted or rejected generally on its quality as determined by the senses of smell and taste. Other tests made quickly, such as the rapid acid test and the temperature, are sometimes used. During the summer months especially, the methylene blue test is resorted to as a means of segregating poor milk.

Several instances came to our attention where producer's milk had been rejected at the receiving plant, although the milk had been promptly and sufficiently cooled to inhibit bacterial growth. The ordinary bacteriological tests to determine the quality of milk, as well as the flavor and odor along with temperature upon arrival, were used as the basis to accept or reject the milk. On checking the methods of production no explanation could be found for the poor quality of milk, but in these cases streptococci of mastitis were always isolated from the producer's supply. When the infected cows were located and their milk withheld, the producer's supply was again accepted at the plant and the quality returned to normal. Also, immediately cooled, sanitarily-produced fresh milk has shown "off-flavors" which were not of feed origin, but were inherent in the milk. Furthermore, in scoring milk from individual cows or separate herds, one frequently encounters a salty flavor which is entirely foreign to normal cow's milk. Formerly such a flavor in the milk was attributed largely to advanced lactation, but lately the salty flavor has been thought to be likewise associated with mastitis. In fact, the salty flavor in milk now arouses suspicion in the

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minds of some milk judges that the milk under examination is from a mastitis-infected cow. In view of these prevailing associations between salty milk, quality of milk, and mastitis infection, an investigation on the subject seemed desirable.

#### METHODS

Samples of milk were obtained from three herds, two of which were known to have mastitis while the third was entirely mastitis-free. The udders of the cows were wiped with a moist cloth, several streams of milk from each quarter were discharged, and the samples collected by milking directly into the sterile containers. The samples were iced immediately so that off flavors due to bacterial growth would not develop.

Upon arrival at the laboratory the methylene blue reduction time and standard plate count were determined on all samples. In addition Breed's smears were prepared and a leucocyte or cell count made and the percentage of chlorides present was determined according to the method of Rosell (6). Cultural examinations, using a differential medium, were made of all samples for the presence of streptococci of mastitis (1). Streptococcal mastitis infection in the cows was established by the presence of the streptococci in the milk and indurations of the udder tissue. Future use of the term mastitis refers to streptococcal mastitis. The samples were then scored organoleptically for flavor and odor, the samples being keyed to prevent during scoring identity of their source and, therefore, possible correlation of flavor. In all 258 samples from 60 cows covering a period of six weeks were studied.

#### RESULTS

*Effect of streptococcal infection upon the chloride content and flavor of milk.*

In preliminary studies some samples of milk had been taken at random from 26 individual cows of a herd which was known to be infected with streptococcal mastitis. The samples were tested organoleptically for salt flavor only. The samples were then identified and the presence or absence of udder infection was recorded.

Eight of the cows from which samples were taken were infected. Of these samples five were definitely salty. Saltiness was not noted in any of the samples from the non-infected animals. All of the cows were yielding a good flow of milk, which may account for the fact that a salty condition was not noted in any of the samples from the non-infected cows. The quantity of the milk yield as well as the extent of infection may have accounted for the fact that saltiness was not noted in some of the samples from the infected cows, although it was noted in the majority of them.

Following this introductory work, studies were made on samples of milk from individual cows of two infected herds and from one non-infected herd

to determine the percentage of chloride present and also to ascertain the flavor quality with special reference to salty flavor. The data in Table 1 represent a six-weeks' study of the milk for chlorides from Herd A, composed of Holstein cows of which 40 per cent showed streptococcic infection.

TABLE 1  
*A comparison of the chloride content of milk from non-infected and that from infected cows*

HERD A

DATE	NON-INFECTED COWS (11-12)				INFECTED COWS (8)			
	No. of samples showing per cent Cl. between			Average Cl. content (%)	No. of samples showing per cent Cl. between			Average Cl. content (%)
	0 - .14	.14 - .16	.16 +		0 - .14	.14 - .16	.16 +	
3/27	0	4	8	.180	0	0	8	.206
4/3	1	5	5	.162	0	4	4	.173
4/10	3	5	3	.151	2	2	4	.171
4/17	0	3	8	.179	0	3	5	.186
4/24	0	7	4	.164	0	3	5	.214
5/1	0	5	6	.168	0	1	7	.204
Total	4	29	34		2	13	33	
Per cent total	6	43	51	.167	3	27	70	.192

Seventy per cent of the individual samples of milk from the infected cows had above the normal limit of 0.16 per cent chloride present, with an average of 0.192 per cent over all samples. The daily chloride content showed a range from an average of 0.171 to 0.206 per cent. Although the average chloride content of the milk from the non-infected cows was considerably lower than that from the infected cows, being 0.167 per cent, it would seem that this was high in light of the figures given for the chloride of normal milk by Hucker (3). Fifty-one per cent of the samples from non-infected cows showed greater than 0.16 per cent chloride, with a daily range from 0.151 to 0.18 per cent.

Similar studies were made on Herd B consisting of Holsteins of which the extent of infection was not so great. The data obtained are presented in Table 2.

The infected samples from this herd were found to have considerably more than 0.16 per cent chlorides. The average was 0.2263 per cent and the daily range was 0.17 to 0.28 per cent. This is much greater than that found in Herd A. The non-infected samples also showed on the average more chlorides present than the non-infected samples from Herd A. Although 53 per cent of the samples from the non-infected cows had less than 0.16 per cent chlorides, while 43 per cent had more than 0.16 per cent,

TABLE 2

*A comparison of the chloride content of milk from non-infected and that from infected cows*

HERD B

DATE	NON-INFECTED COWS (11-13)				INFECTED COWS (1-3)			
	No. of samples showing per cent Cl. between			Average Cl. content (%)	No. of samples showing per cent Cl. between			Average Cl. content (%)
	0-.14	.14-.16	.16+		0-.14	.14-.16	.16+	
3/27	0	5	6	.1691	0	0	1	.23
3/29	0	3	8	.1809	0	0	1	.27
4/3	0	7	4	.1641	0	0	1	.27
4/5	2	2	9	.1700	0	0	1	.20
4/10	1	6	6	.1669	0	0	1	.17
4/17	0	6	7	.1861	0	0	1	.23
4/19	1	8	4	.1746	0	0	1	.19
4/24	0	10	1	.1533	0	0	1	.28
5/1	0	10	1	.1563	0	0	3	.2166
Total	4	57	46		0	0	11	
Per cent total	3.7	53.3	43.0	.1694	0	0	100	.2263

the average from all samples was 0.1694 per cent with a daily range during the six weeks' period from a low of 0.1533 to a high of 0.1861 per cent.

Chloride determinations were made also of samples of milk from individual cows from a Guernsey herd negative to mastitis for 36 months. These data are presented in Table 3.

The average chloride content of the 35 samples was 0.1374, with a range from a low of 0.11 to a high of 0.19 per cent. Of the 35 samples only three had greater than 0.16 per cent chloride, being 0.17, 0.18, and 0.19 per cent. The cows from which these samples were taken were practically dry. A comparison of data in Tables 1, 2 and 3 shows a materially lower per cent of chlorides in the milk from a non-infected herd than that from non-infected cows of an infected herd.

In view of these data, the flavor and flavor scores of the same milk as was used in determining the chloride content is of interest. The data obtained from scoring the samples of milk from the various herds are presented in Tables 4, 5, and 3.

Of the infected samples from Herd A, 56 per cent were criticized for being "salty" and of the non-infected samples from the same herd 25 per cent were criticized for "salty" flavor.

Only one cow in Herd B consistently showed streptococcic infection, although two additional cows, recently freshened, were likewise infected. Samples from these infected cows were criticized for "saltiness" in 90.9

TABLE 3

*The per cent chlorides, flavor score, and flavor criticism of samples of milk from individual cows of a non-infected herd, negative for 36 months*

NO. OF COW	CHLORIDES (%)	CLASS OF MILK*					SCORE	CRITICISM
		Excel.	Good	Fair	Poor	Bad		
1	.12		+				22	
2	.14	+					23	
3	.14		+				21	sl. cowy
4	.12	+					23	
5	.15	+					23	
6	.13		+				22	
7	.11		+				22	
8	.13			+			21	sl. flat
9	.14		+				22	
10	.12	+					23	
11	.14			+			20	flat
12	.14		+				22	
13	.15	+					23	
14	.14		+				22	
15	.15			+			19	salty
16	.14		+				22	
17	.12		+				21	sl. feedy
18	.16			+			20	sl. salty
19	.14		+				22	
20	.12	+					23	
21	.13			+			18	salty
22	.14		+				21	sl. feedy
23	.14		+				22	
24	.12	+					23	
25	.13	+					23	
26	.11		+				22	
27	.17			+			18	salty
28	.12		No samples taken					
29	.11		"	"	"			
30	.15		"	"	"			
31 (dry)	.19		"	"	"			
32	.16		"	"	"			
33 (almost dry)	.18		"	"	"			
34	.14		"	"	"			
35	.12		"	"	"			
Average	0.1374							

\*Key: Excellent, score 23-25  
 Good, " 21-23  
 Fair, " 18-21  
 Poor, " 12-18  
 Bad, " 0-12

per cent of the cases, whereas 20.5 per cent of the non-infected samples and only 14.8 per cent of the samples from the negative herd were similarly criticized.

Not all samples from infected cows were criticized in each case for salty flavor, but the majority of them were as shown by the above figures. Just as the per cent of chlorides present varied so did the intensity of flavor.

Apparently there existed some relationship between the naturally high

in Table 6 where the per cent chlorides, numerical flavor score, and flavor criticism are respectively given of weekly samples of milk from infected cows.

The daily variation in chloride content and flavor of infected samples taken weekly over a period of six weeks is presented in Table 6.

It seems from the data presented in Table 6 that when milk from an infected cow showed a consistently high chloride content that the milk was consistently criticized for being salty, or very salty. On the other hand, when there existed a fluctuation and wide variation in per cent chlorides the flavor was not consistently salty, and furthermore, the salty flavor criticism did not always coincide with the per cent chloride present.

*Effect of streptococcic infection upon bacteriological quality of milk.*

At the same time that the milk samples were collected for organoleptic examination and determination of the chloride content, cow composite and individual quarter samples were collected for bacteriological examination. The methylene blue reduction test was run, bacteria count determined, and a leucocyte count made; also each sample was cultured for streptococci. These results are classified according to the recognized standards or counts.

The results from examination of the non-infected milk samples are given to permit a comparison of the infected and non-infected samples. These data are presented in Table 7.

The number of non-infected cows in Herds A and B are indicated in the Table. It should be kept in mind that these are non-infected cows present in infected herds. In Herd A all except one of the non-infected samples were of class one methylene blue milk. This one was class two at the time of the first examination. The cow concerned was near the end of her lactation period. Two of the samples from Herd B were of class two at the time of the first examination, following which all were consistently class one. All samples from Herd C were class one; this herd had been mastitis-free for a period of 36 months. The total number of samples examined is given in Table 7, as well as those falling in the various classes according to the tests employed. On the basis of the methylene blue test 98.5 per cent of all samples were class one and the remaining one and one-half per cent were class two. No class three or four milks were obtained from non-infected cows.

These samples were further classified according to the numbers of leucocytes per cubic centimeter. In a study of the relation of streptococci infection to udder induration Hucker and Udall (4) established the normal value of 500,000 or less per cubic centimeter. Other workers (5) (2) have set the normal value at 1,000,000 or less per cubic centimeter. Recognizing the value of these standards, the milk samples were classified on this basis.

All of the samples from Herd A contained fewer than 1,000,000 leucocytes per cubic centimeter; of these only six contained more than 500,000 at any examination during the six weeks' period of this study. One sample

TABLE 7  
Composite milk samples of non-infected cows present in *streptococcus infected herds*

DATE	NO. OF COWS	METH. BLUE CLASS				LEUCOCYTES PER CC.				BACTERIA PER CC.			
		1	2	3	4	less than 500,000	500,000 to 1,000,000	more than 1,000,000	less than 100	100 to 200	200 to 500	500 to 1000	more than 1000
3/27	12	11	1	0	0	9	3	0	3	0	3	4	2
4/3	11*	11	0	0	0	9	2	0	1	2	2	5	1
4/10	11	11	0	0	0	10	1	0	0	1	2	7	1
4/17	11	11	0	0	0	11	0	0	0	0	4	6	1
4/24	11	11	0	0	0	11	0	0	0	0	7	4	0
5/1	11	11	0	0	0	11	0	0	0	0	4	7	0
*1 cow ended lactation period													
3/27	11	9	2	0	0	10	1	0	0	2	2	7	0
3/29	11	11	0	0	0	11	0	0	0	2	1	3	5
4/3	11	11	0	0	0	11	0	0	1	0	1	8	1
4/5	13*	13	0	0	0	12	0	1	0	0	3	10	0
4/10	13	13	0	0	0	13	0	0	0	0	4	9	0
4/17	13	13	0	0	0	13	0	0	1	0	4	7	1
4/19	13	13	0	0	0	12	1	0	0	0	5	8	0
4/24	11**	11	0	0	0	11	0	0	0	1	5	5	0
5/1	11	11	0	0	0	10	1	0	0	1	5	5	0
**2 cows freshened													
**2 cows ended lactation period													
35	35	0	0	0	0	35	0	0	25	8	3	0	0
Total	209	206	3	0	0	199	9	1	30	16	55	96	12
Per cent of samples		98.5	1.5	0	0	95	4.3	0.7	14.4	7.7	26.3	45.8	5.8

Herd C  
(mastitis-free)

Herd B

Herd A

from Herd B contained leucocytes in excess of 1,000,000 per cubic centimeter and three samples had counts in excess of 500,000 but less than 1,000,000 at any examination. The remaining milks from Herds A and B, and all samples from Herd C contained less than 500,000 leucocytes per cubic centimeter. The distribution of the samples, on the basis of leucocyte content, indicates that counts of over 1,000,000 per cubic centimeter were not very frequent in the milk from the non-infected cows. In fact the tendency was for the leucocyte content to be much lower than 1,000,000, as evidenced by 95 per cent of the samples falling in the class with less than 500,000 leucocytes, 4.3 per cent in the class from 500,000 to 1,000,000 and only 0.7 per cent of the samples containing leucocytes in excess of 1,000,000 per cubic centimeter.

The bacteria count of the samples varied from one sampling to the next. As indicated in Table 7, approximately 50 per cent of all samples from the non-infected cows contained less than 500 bacteria per cubic centimeter. In addition 45.8 per cent of the remaining milk samples contained between 500 to 1,000, and only 5.8 per cent over 1,000 bacteria per cubic centimeter.

The values obtained upon examination of milk from the non-infected cows present some basis of comparison for the milk from the streptococcus-infected cows. The milk from the infected cows in Herds A and B was studied; these results are summarized in Table 8.

A total of 57 streptococcus-infected milk samples were studied. Upon comparing the percentage of infected samples occurring in the various classes, it is noted that 52.6 per cent of the samples were class one methylene blue as compared to 98.5 per cent of the non-infected samples. A definite decrease in the quality of streptococcus-infected milk is evident, as determined by the methylene blue test, since 28 per cent were class two, 15.9 per cent class three, and 3.5 per cent class four as compared to the 1.5 per cent of non-infected samples lower than class one.

Similarly, the leucocyte content of the infected milk was increased. Fifty-three and seven-tenths per cent of the samples contained over 1,000,000 leucocytes per cubic centimeter and 20 per cent had counts above 500,000 but lower than 1,000,000 with only 26.3 below 500,000 per cubic centimeter. The increase in leucocytes is rather marked when these values are compared with those from the non-infected samples (Table 7).

The bacteria count of the milk from the individual cows varied from day to day, as evidenced by the shift in counts of many of the samples. Of the infected samples 7 per cent had counts between 200 and 500, 24.5 per cent from 500 to 1,000, and 68.5 per cent contained more than 1,000 bacteria per cubic centimeter. Only 5.8 per cent of the non-infected samples had bacteria counts of more than 1,000 per cubic centimeter. It is evident that streptococcus infection of the udder caused a marked increase in the total number of bacteria present in the milk produced.

TABLE 8  
*Composite milk samples of streptococcus infected cows present in herds A and B*

DATE	NO. OF COWS	METH. BLUE CLASS				LEUCOCYTES PER CC.				BACTERIA PER CC.					
		1	2	3	4	less than 500,000	500,000 to 1,000,000	more than 1,000,000	less than 100	100 to 200	200 to 500	1000 to 5000	more than 1000		
Herd A															
3/27	8	5	2	1	0	2	4	2	0	0	1	1	6		
4/3	8	5	0	1	2	2	0	6	0	0	1	1	6		
4/10	8	7	1	0	0	4	0	4	0	0	0	5	3		
4/17	8	8	0	0	0	2	3	3	0	0	2	2	4		
4/24	8	3	1	4	0	2	2	4	0	0	0	2	6		
5/1	8	2	5	1	0	3	2	3	0	0	0	3	5		
Herd B															
3/27	1	0	1	0	0	0	0	1	0	0	0	0	1		
3/29	1	0	0	1	0	0	0	1	0	0	0	0	1		
4/3	1	0	0	1	0	0	0	1	0	0	0	0	1		
4/5	1	0	1	0	0	0	0	1	0	0	0	0	1		
4/10	1	0	1	0	0	0	0	1	0	0	0	0	1		
4/17	1	0	1	0	0	0	0	1	0	0	0	0	1		
4/19	1	0	1	0	0	0	0	1	0	0	0	0	1		
4/24	1	0	1	0	0	0	0	1	0	0	0	0	1		
5/1	1	0	1	0	0	0	0	1	0	0	0	0	1		
Total	57	30	16	9	2	15	11	31	0	0	4	14	39		
Per cent of samples		52.6	28	15.9	3.5	26.3	20	53.7	0	0	7	24.5	68.5		

TABLE 9  
*Results of examination of milk from infected and non-infected quarters of the streptococcus infected cows in Herd A*

DATE	NO. OF QUARTERS	METH. BLUE CLASS				LFCOUNTS PER CC			BACTERIA PER CC.				
		1	2	3	4	less than 500,000	500,000 1 000 000	more than 1,000,000	less than 100	100 to 200	200 to 500	500 to 1000	more than 1000
Examination of milk from the streptococcus infected quarters													
3/27	14	8	1	2	3	1	8	5	0	0	0	3	11
4/3	16	12	1	3	0	4	0	12	1	0	1	5	9
4/10	16	14	1	1	0	4	5	7	2	1	0	4	9
4/17	16	16	0	0	0	5	4	7	0	1	2	1	12
Total	62	51	3	6	3	14	17	31	3	2	3	13	41
Per cent of samples ..		80.8	4.8	9.6	4.8	23	27	50	4.8	3.2	4.8	20.9	66.3
Examination of milk from the non-infected quarters													
3/27	13	15	3	0	0.	9	7	2	2	0	7	4	5
4/3	16	15	0	1	0	8	2	6	2	1	1	4	8
4/10	16	16	0	0	0	11	2	3	4	0	0	4	8
4/17	16	16	0	0	0	8	5	3	0	1	4	3	8
Total	66	62	3	1	0	36	16	14	8	2	12	15	29
Per cent of samples		94	4.5	1.5	0	54.5	24.2	21.3	12.1	3	18.1	22.8	56.1

The fact that a cow may have a streptococcus infection in one, two, three or all four of her quarters raised the question concerning the quality of the milk secreted by the various quarters. In order to make this study, individual quarter samples of milk were collected from the infected cows in Herds A and B. The results of examination of these infected and non-infected quarters are summarized in Table 9.

The methylene blue rating of the milk from the infected quarters is lower than that from the non-infected quarters. Only 80.9 per cent of the infected quarters secreted class one milk, as compared to 94 per cent of the non-infected quarters. Ninety-four per cent of the non-infected quarters of infected cows produced class one milk, as compared to 98.5 per cent of non-infected cows producing class one milk. The per cent of class two milk from both infected and non-infected quarters was quite constant, being 4.8 and 4.5. Only 1.5 per cent of the non-infected quarters was class three and none class four, while the infected quarters yielded 9.6 per cent of class three and 4.8 per cent of class four milk.

The leucocyte counts presented somewhat the same picture with approximately the same percentage of infected and non-infected samples ranging between 500,000 and 1,000,000 per cubic centimeter. These values are 27 and 24.2 per cent, respectively. The infected milks showed a decrease in the percentage of samples with less than 500,000 and a like increase in the percentage of samples with leucocyte counts in excess of 1,000,000 per cubic centimeter.

The data indicate that the infected quarters yield milk of higher bacteria count than that of non-infected quarters. Sixty-six per cent of infected quarters secreted milk containing more than 1,000 bacteria per cubic centimeter as compared to 56.1 per cent of the non-infected quarters. These results seem to indicate that the infection in the involved quarters may exert some influence on the milk secreted by the non-infected quarters, since an increased number of streptococcus-free quarters gave a milk of lower quality than the milk produced by mastitis-free cows.

In order to bring out more clearly, the relationship of streptococcus infection of the udder to the quality of milk produced the data are summarized in Table 10. The percentage of cow composite and quarter milk samples from both infected and non-infected cows that fall into the various classes of the tests employed are presented.

These data indicate that the streptococcus-free cows produced milk of the highest quality as determined by the methylene blue test, leucocyte count, and bacteria count, with the milk from the non-infected quarters of infected cows of next highest quality and the milk from infected quarters and streptococcus infected cows of lowest quality.

The producer's milk supply, as it arrived at the receiving plant, was also studied during the time of the investigation. The quality of the whole

TABLE 10  
*The percentage of milk samples studied that occurred in the various classes of the tests employed*

SAMPLES	METH BLUE CLASS				LFLCOCYTES PER CC				BACTERIA PER CC			
	1	2	3	4	less than 500 000	500 000 to 1 000 000	more than 1 000 000		less than 500	500 to 1000	more than 1000	
Cow § (Infected Non-composite)	52.6	28	15.9	3.5	26.3	20	53.7		7	24.5	68.5	
(infected)	98.5	1.5	0	0	95	4.3	0.7		50*	45.8	5.8	
Quarter (Infected Non-infected)	80.8	4.8	9.6	4.8	23	27	50		12.8	20.9	66.3	
§ (infected)	94	4.5	1.5	0	54.5	24.2	21.3		33.2	22.8	56.1	

§ All figures represent percentage of samples occurring in the classes.

\* Of these 14.4 per cent had counts of less than 100 bacteria per cc. of milk

supply was consistently low during the time that the milk from the streptococcus-infected cows was included. When the milk from the infected cows was withheld the quality of the producer's supply was greatly improved. This is very important when dairymen are desirous of producing a high quality milk.

#### SUMMARY

Samples of milk were secured from individual cows from three herds, two of which showed streptococcic infection while the third showed no streptococcic infection. These samples were tested, organoleptically for salty flavor and for per cent chlorides. Fifty-six per cent and 90.9 per cent of the infected samples from the two infected herds were criticized for having a salty flavor. Twenty-five per cent and 20.5 per cent of the non-infected samples from the infected herds were found to have a salty flavor, as compared to 14.8 per cent of the samples from the negative herd, these latter being from cows almost dry or at least far advanced in lactation.

The averages of the per cent chlorides in the infected samples were 0.192 and 0.2263 for the two herds studied, with the non-infected samples of the same herds averaging 0.167 and 0.1694 per cent, respectively. The milk from the non-infected herd averaged only 0.1374 per cent chloride.

Milk from the infected cows of the two herds scored averages of 19.54 and 18.1 points while the milk from the non-infected cows of the same herds scored averages of 20.67 and 21.17, respectively. The milk of the non-infected herd averaged a score of 21.59.

Although the flavor of mastitis milk was not always salty, the majority of the samples were so criticized. Factors such as stage of lactation, milk yield, and extent of infection, undoubtedly have a marked influence upon the salty flavor of the milk.

The methylene blue test was run on streptococcus-infected and non-infected cow composite and quarter samples of milk. In addition, the leucocyte and bacteria counts of all samples were determined. The milk from the streptococcus-free cows was of higher quality, as determined by the methylene blue reduction test and the number of leucocytes and bacteria per cubic centimeter. The next highest quality of milk was produced by the non-infected quarters of streptococcus-infected cows.

A streptococcus infection in one, two or three quarters of a cow seemed to influence the quality of milk secreted by the non-infected quarters of the same cow. On this account the milk produced by non-infected cows was of higher quality than that secreted by the non-infected quarters of a streptococcus-infected cow.

The infected cow composite and quarter samples of milk were of lowest quality on the basis of the tests employed. They were of lower methylene blue class milk and contained the highest number of leucocytes and bac-

teria per cubic centimeter of milk. These facts are most important when dairymen are interested in the production of high quality milk.

The elimination from the producer's supply of milk from the streptococcus-infected cows in two herds resulted in an increase in the quality of the producer's milk.

Although most of the milk from streptococcus-infected cows had a salty flavor, high chloride content, high leucocyte and bacteria counts, and was of lower quality as determined by the methylene blue test, none of these determinations can be used alone to make an accurate diagnosis of streptococic mastitis.

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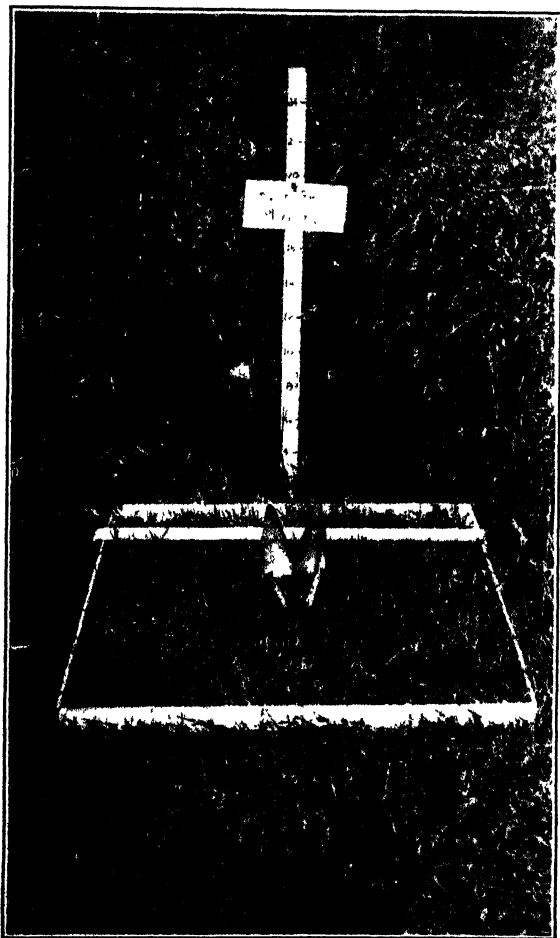
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# A METHOD OF HARVESTING SAMPLES OF PASTURE FORAGE

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A simple device for use in the harvesting of small samples of pasture forage was made from strap iron  $1\frac{1}{2}$  inches in width and  $\frac{3}{16}$  inch in thickness. The iron was shaped into a rectangular frame 20.79 inches by 20.79 inches inside measurement. A separate bar of the same material fits loosely over the frame. The ends of the bar turn downward to keep it in place. (Fig. 1.) The area enclosed by the frame is 3 square feet.



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After selecting the area to be harvested, the frame is held in a horizontal position and dropped into place. When the crop is tall, some of the plants are likely to be bent over by the frame. These may be straightened and those which should be within the frame and those which should be outside may be adjusted to their proper positions by drawing the index and middle fingers along the inside and outside of the frame. A sheep shears is used for cutting the forage. Before the removable bar is put into place, a "swath" is cut by resting the base of the blades on the edge of the frame next to the operator. After the removal of the first swath, the metal bar is laid on the frame and the cutting completed by moving the bar forward as needed, at all times resting the shears on the bar. When the forage is of sufficient length, the harvest may be facilitated by an assistant who grasps a bunch of the forage, holds it during cutting and lifts it out of the way of the next stroke of the shears.

This method accomplishes several purposes: a rigidly-defined area is harvested; the position of the frame does not change during the collection of the sample because of the weight of the device; the forage is cut without scattering the sample, an accomplishment which is difficult when a hand sickle is used; the forage is cut at a uniform height from the ground; the sample is of convenient size for drying.

# THE VITAMIN A ACTIVITY OF BUTTER PRODUCED BY COWS FED ALFALFA HAY AND SOYBEAN HAY CUT IN DIFFERENT STAGES OF MATURITY\*

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The production of butter of high vitamin A value is largely dependent on the vitamin A activity of the feeds fed the cows. The chief sources of vitamin A in the rations of cows are pasture grasses and various types of hay. Hilton, Hauge and Wilbur (1) found that, although the vitamin A activity of butter produced under winter feeding conditions declines when the cows receive hay of low vitamin A value such as timothy hay, it was possible through the feeding of good quality alfalfa to produce butter equal in vitamin A value to that produced when the cows were on pasture. It was also found that soybean hay was less effective in maintaining this high vitamin A activity of butter than was alfalfa hay.

With this close correlation between the vitamin A value of hay fed the cows and the vitamin A value of the butter produced, it is highly essential that hay of high vitamin A activity be fed to dairy cows in order to produce butter of high vitamin A value. However, hays vary considerably in their vitamin A value. This variation may be due not only to differences in the species of the plants from which the hay is produced, but also to the maturity of the plants when harvested and to the conditions of the curing process. Numerous investigators (2-5) have shown that the drying of alfalfa by means of mechanical driers tends to preserve the vitamin A value while the field curing process is more or less destructive to the vitamin A value. Recently, Hauge (6) has shown that the vitamin A value of alfalfa varies with the stage of maturity of the plants, young alfalfa (10-12 inches high) possessing greater vitamin A value than plants in the full bloom stage.

Therefore, it seemed desirable to extend these studies to include a comparison of the vitamin A activity of soybean and alfalfa hay, cut at different stages of maturity and cured by two different processes; and, to study the relationship between the vitamin A activity of the hays and the butters produced by cows fed these hays.

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## EXPERIMENTAL

*The Hay Samples*

The samples of hay used in these experiments were prepared from alfalfa and soybean plants harvested in two different stages of maturity. Portions of each cutting of each hay were field cured and artificially dried. Eight samples of hay were prepared and classified according to the time of harvesting and method of curing.

F.C.Y.A.—Field cured young alfalfa prepared from plants when they were from 10–12 inches high and before the bloom stage.

A.D.Y.A.—Artificially dried young alfalfa hay prepared from plants when they were from 10–12 inches high, before the bloom stage and cured in a mechanical hay drier.

F.C.L.A.—Field cured late alfalfa hay prepared from plants cut at the usual period in the bloom stage.

A.D.L.A.—Artificially dried late alfalfa hay prepared from plants cut at the usual period, in the bloom stage and cured in a mechanical hay drier.

F.C.Y.S.—Field cured young soybean hay prepared from plants 12–15 inches high when the pods were just starting to form on the lower branches.

A.D.Y.S.—Artificially dried young soybean hay prepared from plants 12–15 inches high, when the pods were beginning to form on the lower branches, and cured in a mechanical drier.

F.C.L.S.—Field cured late soybean hay prepared from plants after the beans were well formed in the pods, but with most of the leaves retained and still green.

A.D.L.S.—Artificially dried late soybean hay prepared from plants after the beans were well formed in the pods, with most of the leaves retained and cured in a mechanical drier.

Representative samples of these eight types of hays were collected, finely ground in a Wiley Mill and then subjected to biological assays. The technique employed for the vitamin A assays was the same as has been previously described (6). The results of these tests are given in Table 1, the values being expressed in Sherman and Munsell (7) vitamin A units.

*The Effect of the Hays on Butterfat*

Four Guernsey cows divided into two groups of two cows each were used in these feeding experiments. All cows were in the early stages of lactation at the beginning of the feeding trials, and continued through the tests in fairly uniform production. The two groups were fed the respective samples of hay in successive 30 day feeding periods. Group 1 received artificially dried young alfalfa hay (A.D.Y.A.), field cured late soybean hay (F.C.L.S.), artificially dried late soybean hay (A.D.L.S.) and field cured young alfalfa

hay (F.C.Y.A.) in the order named. Group 2 received in order, artificially dried young soybean hay (A.D.Y.S.), field cured late alfalfa hay (F.C.L.A.), artificially dried late alfalfa hay (A.D.L.A.), and field cured young soybean hay (F.C.Y.S.) during the four successive 30 day feeding periods.

The hay was fed daily at the rate of 1.5 per cent of the body weight of the cows. However, some of the cows failed to consume all of the hay offered and as shown in Table 1, there was some variation in the average daily consumption of the different hays. In addition to the hays, the cows were fed limited amounts of corn silage and a balanced grain ration of ground white corn, ground oats and linseed oil meal.

At the end of each feeding period, composite samples of milk (equal amounts from each cow) from each group were collected, and the cream separated and churned into butter. The butter samples were kept in cold storage and portions removed as needed for biological assays.

The vitamin A activity of these samples of butter were determined by biological assays, using the curative method. The results of these assays are shown in Table 1.

TABLE 1

*Showing the vitamin A values of alfalfa and soybean hays and the vitamin A activity of butters produced by cows fed the respective hays*

	HAY				BUTTER		
	Cow consumption lbs. daily	Vitamin A units		LBS. BUTTERFAT PER DAY	Vitamins A units		
		Per gram	Daily intake (000)		Butter per gram (assayed)	Butterfat per gram (calculated)	Daily output (000)
F.C.Y.A.	12.3	18	100	1.18	29	35	19
A.D.Y.A.	9.7	90	396	1.15	45	55	29
F.C.L.A.	11.5	20	104	1.11	28	34	17
A.D.L.A.	12.8	70	406	1.02	36	43	20
F.C.Y.S.	15.0	36	245	1.06	34	40	19
A.D.Y.S.	10.4	54	254	1.15	38	46	24
F.C.L.S.	12.5	8	47	1.23	19	23	13
A.D.L.S.	16.0	30	219	1.24	24	29	16

## DISCUSSION

In the studies on the vitamin A value of the eight samples of hay prepared for these experiments, it may be seen from Table 1 that the vitamin A value of each artificially dried hay was higher than that of the corresponding field cured sample. It may also be noted that in each case the hays prepared from the younger plants were superior to those made from the more mature plants, except in the case of the field cured young alfalfa which apparently had suffered greater vitamin A destruction during the field curing process than the more mature hay. The lower vitamin A value of the field cured young alfalfa as compared with the field cured late alfalfa hay may

be ascribed to differences in the atmospheric conditions during the curing period. The high humidity encountered during the curing of the young alfalfa undoubtedly retarded the rate of dehydration of the hay and favored the greater enzymatic destruction of the vitamin A value of this hay as compared to that of the late alfalfa (3, 6, 8).

Although the marked differences between the artificially dried and field cured samples of alfalfa are as might be expected, based on previous work reported on this subject (2-5), the proportionate destruction of vitamin A in the young soybeans during the field curing process was considerably less than that of the young alfalfa hay. This presents the possibility that the soybeans contain less enzymes than alfalfa which are destructive to the vitamin A activity of hay during the curing process. In the field cured late soybean hay, the proportionate destruction was not as great as has been found for alfalfa, even though the enzyme activity was favored by rather slow drying due to the high content of beans and fibrous stems as well as unfavorable atmospheric conditions.

Evidence previously reported (8) showed that by immediately destroying the enzyme activity of the plants by heat such as used in mechanical driers, alfalfa hay may be produced with the retention of most of the original vitamin A value of the plants. On this basis it seems logical to assume that by similar treatment soybean hay should retain most of its original vitamin A value. If this is true, it becomes evident that soybean plants do not contain as great a vitamin A potency as do alfalfa plants. This is seen by comparing the artificially dried early cut soybean hay with the artificially dried young alfalfa hay, as well as by comparing the soybean and alfalfa hays of later cuttings.

When these hays were fed as the chief source of vitamin A in the ration of dairy cows, the butter which was produced by the cows fed the respective hays, possessed a vitamin A potency similar in relationship to that possessed by the hays. Not only were the artificially dried hays superior to the corresponding field cured samples in producing butters of higher vitamin A value, but with one exception, all artificially dried hays were more effective than the best field cured hay in producing butter of high vitamin A activity. This one exception was in the case of the artificially dried late soybean hay. The butter produced by this hay possessed a lower vitamin A potency than did the butter produced by most of the other hays. This low vitamin A potency was not due to a lack of vitamin A intake by the cows. In Table 1, it can be seen that during this period these cows had an average daily vitamin A intake of 219,000 units and produced butter with 24 units, while cows fed field cured young alfalfa hay had an average daily intake of only 100,000 units, but produced butter containing 29 units per gram. Furthermore, it may be seen that with artificially dried late soybean hay the vitamin A intake was nearly as high as that of the cows receiving the two differently cured

samples of early cut soybean hay, yet the butter produced by the cows fed the artificially dried late soybean hay was significantly lower in vitamin A value than the other two butters. This suppressing action of late cut soybean hay on the transference of vitamin A of the hay to the butter was also observed in earlier experiments (1) and later found to be associated with some factor in the beans (9). However, it should be noted that soybean hay made from the young plants and fed to dairy cows produced butter of high vitamin A activity.

It is interesting to note that Gillian, Heilbron, *et al.* (10) found that although artificially dried grass was much superior to field cured grass in maintaining the high vitamin A activity of summer butter through the winter feeding period, it did not produce butter with exceptionally high vitamin A activity.

In comparing the vitamin A potencies of the various butters reported in Table 1, it is noted that the butter produced by the cows when fed artificially dried young alfalfa contained 45 units per gram which was much higher than any of the other samples; in fact, it possessed the highest vitamin A activity of any butter sample ever tested in this laboratory. Apparently by feeding hay of very high vitamin A content it is possible to increase the vitamin A value of butter even above that produced by cows fed good blue grass pasture. The highest vitamin A sample of butter produced by cows on good blue grass pasture and tested in this laboratory contained 36 units per gram.

However, it is apparent that there is a rather definite limit to the possible increase of the vitamin A in the butter regardless of the number of vitamin A units in the ration. In these experiments, for example, there was four times greater daily vitamin A intake with artificially dried young alfalfa hay than with the corresponding field cured sample, yet there was only a 50 per cent increase in the vitamin A value of the butter.

#### SUMMARY

1. Comparisons were made of the vitamin A value of artificially dried and field cured alfalfa and soybean hay, cut at two different stages of maturity. Studies were also made of the relationship between the vitamin A activity of the hays and the butters produced by cows fed these respective hays.

2. Artificially dried hays were superior in vitamin A value to the corresponding field cured hays.

3. Hays made from younger plants possessed a higher vitamin A value than did hays made from older plants.

4. Alfalfa plants contain greater vitamin A potency than do soybean plants.

5. Dairy cows when fed artificially dried hays produced butter of higher vitamin A value than when fed field cured hays.

6. Artificially dried young alfalfa hay when fed to dairy cows produced butter of exceptionally high vitamin A value—45 units per gram.

7. Soybean hay made from plants after the beans were well formed in the pods suppressed the formation of vitamin A in the butter sufficiently to produce a butter of only medium high vitamin A activity. Soybean hay made from young plants and fed to dairy cows did not show this suppressing action and produced butter of high vitamin A activity.

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# THE SOLUBILITY-FREEZING POINT RELATIONSHIPS OF WATER SOLUTIONS SATURATED WITH RESPECT TO SUCROSE AND DEXTROSE IN RELATION TO THE STORAGE OF SHERBET AND WATER ICE

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It has long been known that the crusting of sherbet and water ices is due to the crystallization of cane sugar. Leighton (1) showed that, theoretically, such sugar separation could not take place under ordinary conditions if the sherbet was held at a temperature higher than the eutectic temperature of the system cane-sugar-water, which he determined to be as approximately  $-12^{\circ}\text{C}$ . Dahlberg (2) showed this independently, and also showed that sugar separation could be prevented at even lower temperatures if dextrose or corn sugar was used in place of part of the cane sugar. Experiment showed him that a concentration of 7% dextrose and 25% sucrose was the most satisfactory for the prevention of sugar separation, although a slightly higher proportion of dextrose could be used. With the realization that the greatest protection was to be had if the proportion of the two sugars to water was that of their ternary eutectic mixture, but lacking suitable laboratory facilities for the precise determination of the joint solubility and freezing point relationships of sucrose and dextrose at temperatures below ordinary room temperature, Dahlberg extrapolated from the data then existing for the relationships at higher temperatures and came to the conclusion that the freezing point of a water solution just saturated to both sucrose and dextrose would be about  $-20.0^{\circ}\text{C}$ . and that the proportion of dextrose to sucrose would be approximately that found by him to be most suitable for use in practice, namely, 7 parts dextrose to 25 sucrose, or 1 to 3.57.

While the past few years have witnessed an increased utilization of dextrose in frozen products, the exact solubility and freezing point relationships of water solutions saturated to sucrose and dextrose have not been determined at temperatures below  $30^{\circ}\text{C}$ . This being the case, work was undertaken to determine these relationships, and the data are recorded in this paper.

The procedure best suited for the purpose of determining the eutectic temperature and the composition of the ternary eutectic mixture was to determine the joint solubility in water of corn and cane sugar at a number of temperatures below room temperature, and then to determine the freezing points of a number of these saturated mixtures. The eutectic curve obtained

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by plotting the total sugar concentration against temperature should intersect the freezing point curve obtained from these mixtures at the true ternary eutectic point. In this work the brine temperatures of the refrigeration system were not low enough for the solubilities of cane and corn sugar to be measured at the true eutectic; however, the temperatures obtainable were sufficiently low to permit of extrapolation, and we believe we have located the ternary eutectic temperature with an accuracy of  $\pm 0.1^\circ \text{C}$ .

For the solubility work a thermostat was used which was equipped with brine coils for cooling and an electric heater for maintaining the desired temperature against the cooling action of the coils. Temperatures could be maintained with an accuracy of  $\pm 0.03^\circ \text{C}$ .

For the determination of the joint solubilities of the two sugars, equilibrium was approached from under-saturation by rotating within the thermostat 100 cc. glass stoppered bottles containing sugar solution and an excess of the solid phase of each sugar. Rotation was continued until equilibrium was reached, as shown by the analysis of solution taken at intervals of one week. At the lower temperatures a time interval of six weeks was required for the establishment of equilibrium.

When portions of the liquor were to be withdrawn for analysis, the bottles were placed on a rack on the side of the thermostat, and after the solid phase had had time to settle, the liquor was withdrawn by suction into a pipette, the end of which was flared and covered with one layer of filter paper supported by a small perforated platinum disc. In this manner a true sample of about four grams of liquid was obtained by the filtration of the liquor. The actual analyses by means of density and polarimetric measurement upon the diluted sample were carried out in the manner described by Jackson and Gillis (3) and Jackson and Silsbee (4).

The freezing point determinations were conducted in the usual manner using Beckmann thermometers with ice and alcohol as the refrigerant.

TABLE 1

*The solubility-freezing point relationships of water solutions saturated with respect to sucrose and dextrose*

TEMPERATURE	TOTAL SUGAR	SUCROSE	DEXTROSE	WATER	F.P.
$^\circ \text{C}$ .	<i>per cent</i>	<i>per cent</i>	<i>per cent</i> (anhydrous)	<i>per cent</i>	$^\circ \text{C}$ .
30.0	73.7 <sup>1</sup>	47.1	26.6	26.3	
23.3	72.2	48.4	23.8	27.8	
9.2	69.5	50.7	18.8	30.5	
- 1.3	67.5	51.2	16.3	32.5	-21.20
- 0.5	66.7	51.4	15.3	33.3	-20.10
-12.9	65.7	51.9	13.8	34.3	-18.98
-17.9	64.9 <sup>2</sup>	52.2	12.7	35.1	-17.90

<sup>1</sup> Jackson and Silsbee (4).

<sup>2</sup> By extrapolation.

Great care was taken to standardize the procedure and to prevent an undesirable degree of supercooling. The final figures are each the mean of several determinations.

The data are given in Table 1 and are plotted in Figure 1.

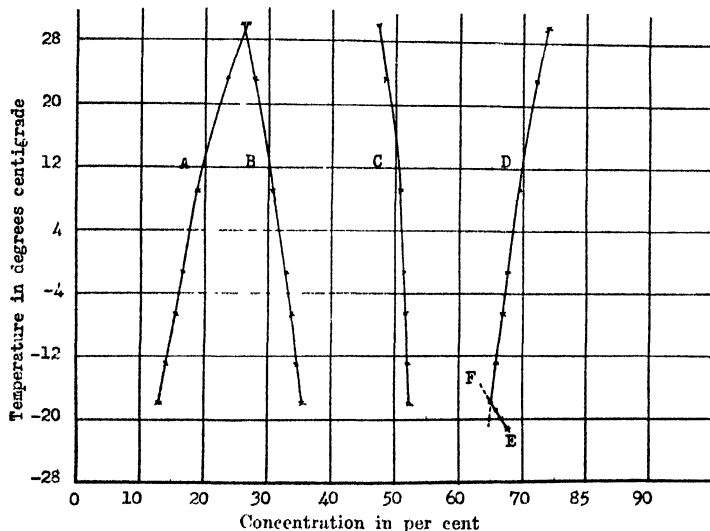


FIG. 1. Solubility-freezing point relationships of water solutions saturated with respect to sucrose and dextrose.

A Dextrose

B Water

C Sucrose

D Dextrose plus sucrose

E Freezing curve

F Eutectic

The ternary eutectic temperature of the system sucrose-dextrose-water is  $-17.9^{\circ}\text{C}$ . and the proportion of dextrose to sucrose is 1 part to 4.11 parts.

It is to be noted that this is a markedly lower proportion of dextrose to sucrose than that indicated as permissible in practice. The conclusion is to be drawn, therefore, that in practice a certain degree of supersaturation is permissible, this amount being indicated by a comparison of these data and the figures presented in the work of Dahlberg (2).

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# THE AGE THICKENING OF SWEETENED CONDENSED MILK

## II. EFFECT OF FOREWARMING CONDITIONS\*

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It is a matter of common experience in the evaporated and sweetened condensed milk industries that forewarming conditions, especially temperature, greatly influences the subsequent stability of the finished product. The usual temperature range of 130 to 145° F. at which the milk is condensed in the pan is not sufficiently high to insure destruction of all disease germs, quality-damaging organisms and enzymes that might be present.

A relatively high forewarming temperature is desirable from a biological standpoint especially where the milk is heated in the presence of the sucrose or remnants of previous batches of sweetened condensed milk. Rice (1) has shown that enzymes are more stable toward heat when in the presence of sucrose and that a rancid flavor is apt to develop unless a rather high forewarming temperature is used. He found that in the absence of sucrose, forewarming to 140° F. for 15 minutes or to 150° F. for an instant was sufficient to destroy lipase activity. The forewarming temperature used is further limited by its effect upon the subsequent thickening of the product. The viscosity must be high enough to prevent fat separation but not so great as to be objectionable from the standpoint of the consumer.

In condensery practice a wide range of forewarming temperatures are used and the milk and sugar may be heated together or the sugar solution may be heated separately. A common practice is to heat the milk and sugar with live steam until it begins to boil and then to start drawing it into the pan. These practices have been subjected to experimental study in this laboratory to determine their effect upon the subsequent age thickening of the finished product.

### EFFECT OF FOREWARMING TEMPERATURE

The experimental procedure used in these trials has been given in a previous paper (2).

Table 1 shows the progressive age thickening of 8 batches of sweetened condensed milk made from the same milk but with different forewarming temperatures. The milk and sugar were heated together for 10 minutes at the temperature indicated and then promptly cooled to 131° F., the temperature of condensing, before drawing the milk into the pan. For the tempera-

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ture below 203° F. the milk was forewarmed in a water bath, at 203° F. the milk was heated directly with steam, and for the temperatures above boiling an autoclave was used.

TABLE 1  
*Effect of forewarming temperature—January 23, 1934*

°F. TEMP.	135	150	165	180	195	203	230	248
<i>Days</i>	<i>Viscosity in Poises</i>							
0	7.4	7.4	8.7	17.3	22.9	19.8	7.4	7.4
2	8.7	7.4	8.7	92.0	*	*	11.55	13.93
6	19.42	16.1	11.55	1584.0			11.55	19.42
11	58.5	19.42	25.8	*			16.75	19.42
16	403.5	13.93	22.3				19.42	22.3
23	403.5	19.42	25.8				19.42	22.3

\* Samples too viscous to measure.

Sucrose 44.1%, Fat 7.95%, Milk solids-not-fat 21.43%.

The results show that forewarming temperatures of 150 and 165° F. make a less viscous product than heating the milk to only 135° F. With temperatures of 180° F. and up to boiling the milk becomes considerably less stable, while temperatures above boiling again make the milk less susceptible to age thickening, but produced a dark-brown discoloration in the finished product, especially after a period of storage at 37° C. With the low forewarming temperatures there is danger of fat separation during storage. The excessively high viscosities of the 195 and 203° F. forewarmed milk are greater than is usually the case but are due to the high milk solids content of the finished product as shown at the bottom of the table

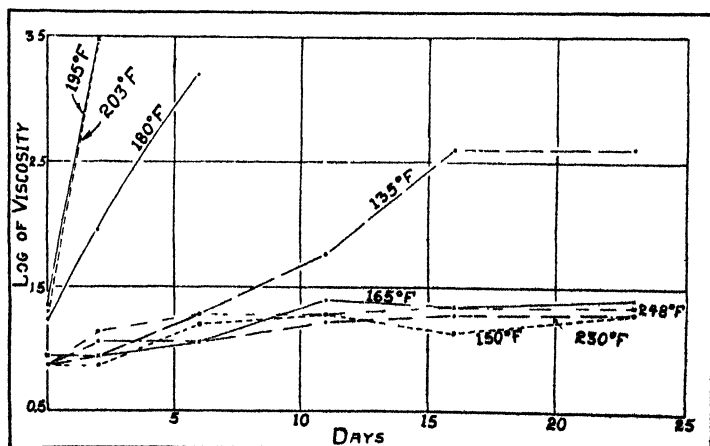


FIG. 1. EFFECT OF FOREWARMING TEMPERATURE ON AGE THICKENING.

Table 1 is plotted in graph 1 and shows that batches made with forewarming temperatures of 150, 165, 230 and 248° F. exhibited about the same tendency toward age thickening.

This trial was repeated with essentially the same results as is shown in Table 2.

TABLE 2  
*Effect of forewarming temperature—February 15, 1934*

°F. TEMP.	135	150	165	180	195	203	242
<i>Days</i>			<i>Viscosity in Poises</i>				
0	6.2	6.2	7.4	8.7	8.7	8.7	7.4
4	6.2	6.2	7.4	33.5	393.3	362.9	39.6
8	13.6	9.9	13.6	192.9	*	*	158.9
14	25.8	13.9	19.4	1072.0			505.1
19	25.8	16.8	25.8	1406.0			717.0
25	53.0	22.3	47.5	*			1850.0

\* Samples too viscous to measure.

Sucrose 44.1%, Fat 8.46%, Milk solids-not-fat 19.48%.

Visible fat separation in the 135 and 150° F. forewarmed batches.

#### EFFECT OF PROLONGED HOLDING PERIOD AT FOREWARMING TEMPERATURE

In these trials the milk and sugar were forewarmed together, held for the period designated, and then quickly cooled to 135° F. before drawing the milk into the pan.

The results given in Table 3 are for stable milk and show that as the length of the holding period increases the tendency to thicken becomes greater. In Table 4 a higher forewarming temperature was used and the milk was originally unstable.

TABLE 3  
*Effect of holding period—February 8, 1934*

	NO HOLDING PERIOD	15 MINUTES	30 MINUTES	60 MINUTES
<i>Days</i>		<i>Viscosity in Poises</i>		
0	7.4	7.4	7.4	7.4
4	19.42	95.0	125.0	252.8
11	22.3	124.1	221.5	282.6
15	25.8	138.8	163.2	218.8
21	25.8	148.5	173.5	293.0

Sucrose 44.10%, Fat 8.05%, Milk solids-not-fat 20.22%.

Forewarming temperature, 190° F.

From these results it appears that the effect of a prolonged holding period depends upon whether or not the milk is originally stable, the effect being to stabilize unstable milk and to unstabilize stable milk toward age

TABLE 4  
Effect of holding period—May 31, 1934

	NO HOLDING PERIOD	15 MINUTES	30 MINUTES	60 MINUTES
<i>Days</i>	<i>Viscosity in Poises</i>			
0	31.0	32.0	7.4	7.4
1. ...	1981.0	*	307.5	403.5
4	*		*	*

\* Samples too viscous to measure.

Sucrose 44.10%, Fat 8.32%, Milk solids-not-fat 20.15%.

Forewarming temperature 203° F.

thickening. However, the effect is too slight to be of any practical significance in attempting to stabilize unstable milk in this manner. Graph 2 shows the effect of the holding period for both stable and unstable milk.

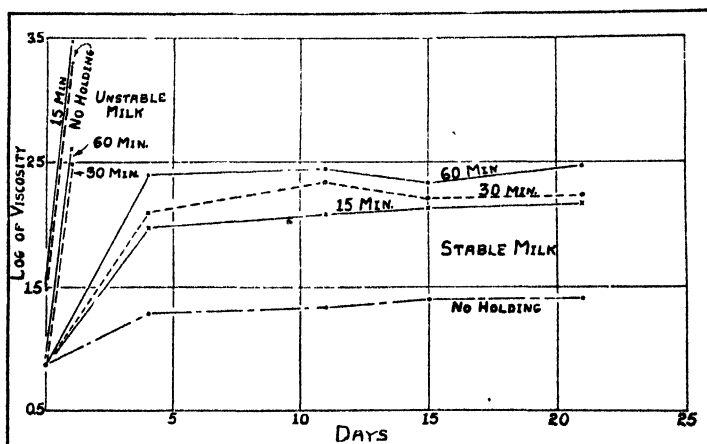


FIG. 2. EFFECT OF HOLDING PERIOD AT FOREWARMING TEMPERATURE ON AGE THICKENING.

#### EFFECT OF HAVING THE SUGAR AND MILK TOGETHER DURING EXPOSURE TO THE TEMPERATURE OF FOREWARMING AND CONDENSING

The effect of having the sugar in contact with the milk during the forewarming and during the condensing is shown in Table 5. In this trial, the effect of forewarming the sugar syrup and milk separately at 185° F. for 10 minutes, cooling to 135° F., and then mixing the two together before drawing into the pan was compared with adding the sugar syrup near the end of the condensing period. These results are plotted in graph 3.

The results show that having the sugar in contact with the milk during the forewarming period has the greatest effect in causing the milk to thicken

TABLE 5  
Effect of sugar on age thickening -March 1, 1934

Days	A		B		C	
			Viscosity in Poyes			
0	7.4		2.76		2.76	
5	78.2		33.5		28.4	
11	120.0		41.8		28.4	
15	114.6		58.5		28.4	
22	192.9		83.9		28.4	

Sucrose 44.1%, Fat 8.36%, Milk solids not fat 20.55%.

A—Sugar and milk forewarmed together.

B—Sugar and milk forewarmed separately, cooled to 135° F., and then mixed together before drawing into the pan.

C—Sugar added as a syrup near the end of the condensing period.

during aging, while having the sucrose in contact with the milk during condensing (131° F. for about 2½ hours) greatly decreases the viscosity, but makes a more viscous product than when the sugar is added near the end of the run. Adding the sugar near the end of the condensing period is likely to produce a product which is too thin and which will show fat separation during storage.

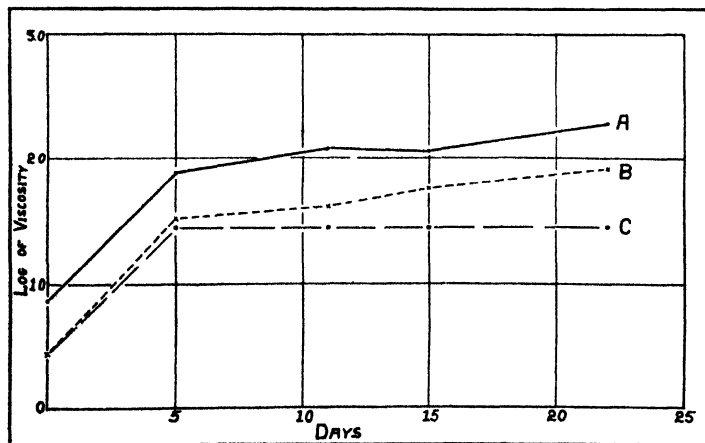


FIG. 3. EFFECT OF SUGAR ON AGE THICKENING.

A—Sugar and milk forewarmed together.

B—Sugar and milk forewarmed separately, cooled to 135° F., and then mixed together before drawing into the pan.

C—Sugar added as a syrup near the end of the condensing period.

#### DISCUSSION

The method of controlling the age thickening by the use of the proper forewarming temperature is quite satisfactory during the season of the year

when the milk is not originally unstable. A temperature of 175 to 185° F. is quite satisfactory from the standpoint of age thickening and is sufficient to destroy lipase activity, especially if the sugar and remnants of previous batches are not added until after the milk is up to temperature. Forewarming temperatures below 165° F. should be avoided as there is too much danger of fat separation.

A 10 minute holding period at forewarming temperature seems to be quite satisfactory. Longer holding periods as a means of stabilizing unstable milk cannot be recommended.

Where sucrose alone is used as the sweetening agent it should not be withheld from the milk until near the end of the condensing period as is done with corn sugar. In every trial in this laboratory where the sucrose was withheld until late in the condensing period the milk was excessively thin and showed fat separation. In cases where the finished product thickens too rapidly it may be satisfactorily controlled by forewarming the milk and sugar separately, cooling to the pan temperature, and then mixing the two before drawing into the pan.

#### CONCLUSIONS

Forewarming temperatures of 150 and 165° F. make a product which thickens less rapidly than heating the milk to only 135° F. With temperatures from 180° F. up to boiling the milk becomes considerably more unstable, while temperatures above boiling again make the milk less susceptible to age thickening.

Forewarming temperatures above boiling cause considerable darkening in the finished product during storage.

Prolonged holding periods at the forewarming temperature tend to destabilize stable milk, but have a slight effect in stabilizing unstable milk.

Having the sucrose in contact with the milk during forewarming has the greatest effect in causing the milk to thicken during storage, while having the sucrose in contact with the milk only during condensing at a temperature of 131° F. greatly decreases the viscosity, but makes a more viscous product than adding the sucrose as a syrup near the end of the condensing period.

Excessive age thickening may be prevented by withholding the sucrose during the time that the milk is at forewarming temperature.

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# CAUSES OF DIFFERENCES IN BUTTERFAT PRODUCTION OF COWS IN IOWA COW TESTING ASSOCIATIONS\*

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It is important to know the extent to which differences between production records are caused by environmental factors and how much they are caused by heredity since it is only the hereditary differences which make possible any permanent breed improvement. The present study was undertaken to measure the relative importance of the chief causes of variation among records of cows in Iowa Cow Testing Associations.

## SOURCE OF DATA

Through the cooperation of herd owners and of members of the Dairy Extension Service staff at Iowa State College, the record books belonging to men who had been members of a Cow Testing Association for at least three consecutive years, some time during the period 1922 to 1932, were sent to the college where the data desired were copied for this study. Besides the amount of milk and the fat percentage at each monthly test the record books contain, with varying degrees of accuracy and completeness, information about the registration number (or barn number in case of grades) and birth date of the cow, registration number of the sire and dam of the cow, date and age of cow when lactation started, dates when cow went dry and date on which the first test in each lactation was conducted.

The merit of the ration fed to each cow was scored according to an arbitrary scale running from 1 to 9. Grade 1 feeding consisted of legume hay, silage, three kinds of grain including bran, protein supplement, grain while on pasture and grain to dry cows, while grade 9 consisted of non-legume hay and one grain fed whole. All scoring was done by Mr. G. G. Gibson of the Dairy Husbandry Extension staff. The various standards for grading the feeding policy in each lactation are shown on p. 812.

The records from 95 different herds of Guernsey, Holstein and Jersey cattle were used for this study. These herds had been tested regularly in a Cow Testing Association for a period which varied from 3 to 11 years, but averaged 6.8 years. The number and kind of cows under investigation are summarized in Table 1.

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\* The writer is indebted to Dr. Jay L. Lush for stimulating suggestions during the conduct of this study and for valuable criticism of the manuscript.

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*Standards for Grading*

GRADE	ROUGHAGE	GRAIN	NOTES
1	Legume hay and silage	3 grains including bran	a
2	“ “ “ “	2 grains	a
	or Legume hay	3 grains including bran	a
3	Mixed hay and silage	“ “ “ “	a
	or Legume hay	“ “ “ “	a
4	Timothy or mixed hay and silage	“ “ “ “	b
	or Timothy or mixed hay and silage	“ “ “ “	c
5	Mixed hay and silage	2 grains	
6	Timothy hay	3 grains including bran	
	or Silage	2 grains	
7	Non legume hay and silage	2 grains (whole)	
	or Silage	2 grains (ground)	
8	Non legume hay	2 grains (whole)	
	or Silage	2 grains (ground)	
9	Non legume hay	1 grain (whole)	

Grades 1 to 6 required also the inclusion of a protein supplement

a Grain fed when on pasture and grain fed to dry cows

b Grain fed when on pasture

c Grain fed to dry cows

TABLE 1

*Number of cows and number of lactations of cows of the different breeds*

BREED	PUREBRED				GRADE				TOTAL			
	Cows		Lactations		Cows		Lactations		Cows		Lactations	
	No	%	No	%	No	%	No	%	No	%	No	%
Guernsey	69	3.0	162	2.8	313	13.5	870	14.9	382	16.5	1032	17.6
Jersey	850	36.7	1859	31.7	721	31.1	2024	34.5	1571	67.8	3883	66.3
Holstein	220	9.5	553	9.4	143	6.2	392	6.7	363	15.7	945	16.1
Total	1139	49.2	2574	43.9	1177	50.8	3286	56.1	2316	100.0	5860	100.0

## ADJUSTMENT OF DATA

The measure of production used in the present study was the total butter-fat production in the first eight monthly tests of all lactations which lasted at least 9 months.\* If the figures thus obtained are multiplied by the aver-

\* This measure was chosen on theoretical grounds as long enough to show some differences in persistency but not long enough to be as seriously influenced by differences in management of C.T.A. herds as 10 month or 12 month lactations would be. The provision

age number of days per month, viz. 30.5, the production for the first eight months of the lactation is obtained.

All records were corrected to a mature equivalent basis using factors obtained from these data. All lactations known to follow abortions were discarded. The record books gave no information concerning the number of times daily the cows were milked, and no corrections could be made for this. In the findings this and other unknown or unrecorded differences in management will appear as causes of variation which in some cases affect all herd mates alike but in other cases will affect only the individual cow.

#### METHODS OF INVESTIGATION

The data were analyzed by the method of analysis of variance described by Fisher (3). By this method, of which an example is shown in Table 3, it is possible to isolate the variance brought into the data from various sources. When the total variance is to be analyzed into its component parts the observations are classified on the basis of certain criteria which it is supposed may be significant causes of variation. After the variance between the classes has been isolated, a residual variance, commonly called "experimental error," will be left. The relation between the total variance and the residual variance can be expressed either as a correlation between the observations within a class, or as that fraction of the variance caused by the factor, or whole complex of factors, which affects alike all member of the same class.

All the data were punched on cards and computed with the Hollerith tabulating machine.

#### RESULTS

##### *General time trend*

During the 11 years included in the present study the average production increased about 50 pounds of butterfat (on a 10-month lactation basis). However, only 2.8 per cent of the total variance is due to changes in yearly averages. Therefore the time trend which the data show, although significant, is one of the minor causes of variation among these records.

##### *Difference between purebred and grade cows*

The average production of all the grade cows (as a total for the first eight monthly testing days) was  $10.70 \pm .04^2$  pounds of fat while the average production of the purebred cows was  $11.63 \pm .06$  pounds of fat

about the ninth month was used to eliminate cases where the cow was almost dry on the last testing day. However later studies have indicated that it would have made little difference if lactation lengths of 9, 10, 11 or 12 months had been used or even if association yearly records had been used.

<sup>2</sup> In this paper the figures after the  $\pm$  are standard errors.

or 8.7 per cent higher. This difference corresponds roughly to a difference of 34 pounds of butterfat for 10 months' production.

This agrees closely with the findings of McDowell (9) who studied over 100,000 yearly records of purebred and grade cows tested in Dairy Herd Improvement Associations. He found that purebred cows excelled grades by 6.7 per cent in production of butterfat but consumed 23 per cent more units of feed than grades. McDowell points out that the higher production of the purebreds might have been due to better feeding.

TABLE 2  
*Average production of purebred and grade cows by breeds*  
(Expressed as total pounds of fat produced in the first eight monthly test days)

BREED	AVERAGE PRODUCTION		DIFFERENCE PUREBRED ABOVE GRADE	STANDARD ERROR OF MEAN DIFFERENCE*
	Purebred	Grade		
Guernsey	10.60	10.42	.18	.23
Holstein	12.08	10.73	1.35	.08
Jersey	10.41	11.13	-.72	.17

\* The standard error of the difference is figured from the standard deviation within breed (see Table 3),  $2.64 \times \sqrt{1/n_1 + 1/n_2}$ .

#### *Variance due to breed differences*

Table 2 shows the averages by breed. The total variance for the grade records is 5.61 while the total variance for the purebreds is considerably higher, viz. 8.50. Both for purebred and grade cows there is a significant difference between breeds. But while 618 per cent of the total variance disappears when breed is held constant in the purebreds, only a little more than one half of one per cent of the variance disappears when the same is done for the grade cows. When purebred and grade cows are analyzed together, breed differences account for 2.09 per cent of the total variance, but practically all of this breed variance is caused by purebred cows.

Apparently purebred cows show distinctly more difference between breeds than grade cattle do, and the division of grade cows into different "breeds" may seem unjustified insofar as butterfat production is concerned. Without attempting to give a complete explanation of this situation, two suggestions may be offered. First, part of this lesser variation among the grade cows may be caused by a more vigorous culling of low producing grades than of low producing purebreds. McDowell's (9) study of the records of purebred and grade cows seems to show that there is a more intense culling for grade cows. However, grade cows included in the present study were in these herds long enough to have an average of 2.79 lactations each while the purebreds have only an average of 2.26 lactations each. Second, some purebreds (but not all) are forced by high feeding, extra milking, etc., into unusually high production. Naturally there would be

less of this among grades and perhaps it is a less frequent practice with purebreds of some breeds than others.

*Variance due to herd differences*

The average production of the different herds varies considerably. The variance within herd is 34.1 per cent less than the intra-breed variance (Table 3). This corresponds to a correlation of .34 between records chosen at random from the same herd in a population of many herds all belonging to the same breed.

TABLE 3  
*Analysis of variance due to breed, herd, and cow*

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	STANDARD DEVIATION
Between breeds	2	877	438.4	
Within breeds	5857	40682	6.946	2.64
Between herds within breed	118	14404	122.1	
Within herds within breed	5739	26278	4.579	2.14
Between cows within herd	2195	16553	7.540	
Between records of the same cow	3544	9725	2.744	1.66
Total	5859	41559	7.093	2.66

All differences are highly significant ( $P < .01$ ).

Portion of total variance due to differences between:

$$\text{Breeds } \frac{7.093 - 6.946}{7.093} = 2.09\%.$$

$$\text{Herds } \frac{7.093 - 4.579}{7.093} = 35.4\%.$$

$$\text{Cows } \frac{7.093 - 2.744}{7.093} = 61.3\%.$$

Portion of intra-breed variance due to differences between:

$$\text{Herds } \frac{6.946 - 4.579}{6.946} = 34.1\%.$$

$$\text{Cows } \frac{6.946 - 2.744}{6.946} = 60.5\%.$$

Portion of intra-herd variance due to differences between cows:

$$\frac{4.579 - 2.744}{4.579} = 40.1\%.$$

Records from the same herd may be either from the same cow, but made in different years, or they may be from different cows and made during the same or in different years. Herd mates may be related or may be somewhat alike because each owner has selected or culled them according to his own ideas. In order to exclude the cases where cows have more than one record, the analysis of variance of the first available records of the 2316 cows was carried out in a manner similar to the one shown in Table 3. The variance due to herd differences was 33.0 per cent of the intra-breed variance. This

corresponds to correlation of .33 between first available records of herd mates. The smallness of the difference between 33.0 and 34.1 is not surprising since two records chosen at random from a large herd would rarely be records of the same cow.

The important herd effect shown by this correlation of about one third may have several causes. Each herd may be a distinct group with a certain uniformity, obtained either by selection or by consanguinity among the animals. Such selection and culling may not have been toward the same ideal nor with equal intensity in all herds. The environment and management may be rather uniform for all cows in the same herd but distinctly different from herd to herd. Gowen (7, pp. 278-298) using Holstein-Friesian Advanced Registry data found that the correlation ratio between herd and milk yield was .603 and the correlation ratio between herd and butterfat percentage was .490. If the degrees of freedom are taken into consideration these correspond to correlations of .33 for milk and .20 for butterfat percentage between records of herd mates. Although Gowen's figures come from Advanced Registry data, they show a herd effect of surprisingly near the same size as was found in these Iowa Cow Testing Association herds.

#### *Variance due to differences between cows*

Table 3 shows that 40.1 per cent of the intra-herd variance and 60.5 per cent of the intra-breed variance are due to differences between cows. (Since in these data all records of a cow were made in the same herd, the intra-breed differences between cows include the herd difference.) This corresponds to a correlation of .40 between records of the same cow in a population of cows all belonging to the same herd, or to a correlation of .60 between records of the same cow in a population of cows kept in many herds. In a single Jersey herd Gowen (5, 6) found an average correlation of .54 and .52 respectively, between milk production and fat percentage of one lactation at any age, and milk production and fat percentage of another lactation at another age. In a population of cows from many herds Gowen (7) found an average correlation of .67 between successive 365-day milk yields of Holstein-Friesian cows and an average correlation of .70 between similar yields of Guernsey cows. Likewise in a population of Danish cows from many herds, Gaines and Palfrey (4) found for yield of fat-corrected-milk (milk energy) an average correlation between successive lactations of .50. Each of those cows had been kept long enough to have ten normal lactations. Probably few really low producers among those which started on test would have escaped culling this long. The correlation of .60 found in the present study is somewhat higher than the average correlation found by Gaines and Palfrey, but somewhat lower than the correlation found by Gowen.

Breed, herd, and cow differences together account for 61.3 per cent of

the total variance. The remaining 38.7 per cent of the variance is due to differences between the records of the same cow. These differences may be caused by a number of factors, such as year to year changes in weather, management and feeding, length of preceding dry period, season of calving, imperfections in the corrections for age, errors in assuming that the actual production during the eight months was accurately shown by the monthly tests, and perhaps other factors. Even after allowance has been made for feeding, length of dry period, season of calving and other recorded factors of management, there will be a considerable residual variance left, the cause of which cannot be determined precisely. For convenience such causes may be lumped together as unrecorded or unknown variations in the environment.

### *Influence of feeding*

The feed the cows received was scored according to an arbitrary scale planned to run from 1 to 9, but only two cows' feeding actually was scored lower than grade 7. Cows receiving grade 1 feeding produced approximately 490 pounds of butterfat for a 10-month period while cows receiving grade 7 feeding produced only about 276 pounds of butterfat. The analysis of variance shows that there are significant differences between the production of cows grouped in the different feed grades. When the differences in average production which are associated with differences in feed grade are eliminated the variance is reduced 16.8 per cent. However the correlation between feed grades of the same cow in consecutive years is .78. This correlation is made up of a correlation of .92 between means of herds and a correlation of .59 within herds. Therefore there are marked differences in the feeding policy from herd to herd and there is some tendency to feed the same cow the same way year after year. This indicates that a considerable part of the differences which exist between herd averages is associated with recorded variations in their feeding practices. Doubtless there were other important but unrecorded differences in feeding practices. Of the intra-herd variance 8.3 per cent (which equals 5.4 per cent of the total variance) was due to variations in feeding grade. The variance associated with feeding grade (16.8 per cent) may then be divided into one part (5.4 per cent) due to unlikenesses in feeding within herds and another part (11.4 per cent) due to differences in the feeding policy from herd to herd.

The condition of the cow at calving is usually considered to be an important factor in determining her production, at least during the first part of the lactation. An analysis of variance shows that the feed grades of the previous year and that of the year the record is made, together are responsible for 1 per cent more of the total variance than is the feed grade the year the record is made. Apparently there is some after-effect of the previous year's feeding, so far as that is different from the current year's

feeding, but this effect is small compared with the total variation when allowance has already been made for the feeding the year the record is made.

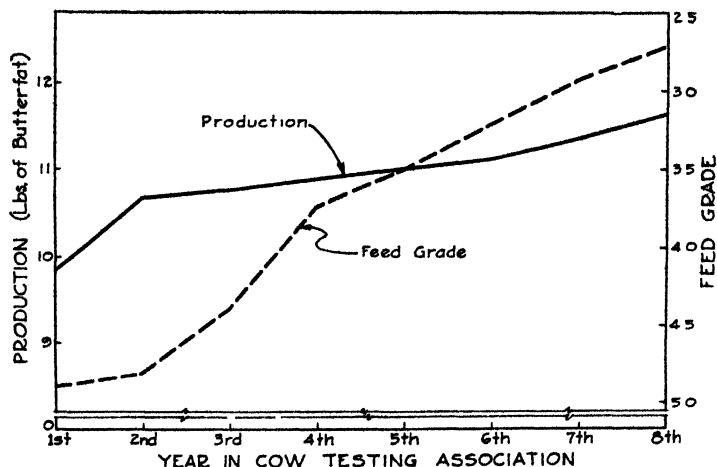


FIG. 1. AVERAGE PRODUCTION AND AVERAGE FEED GRADE FOR COWS IN HERDS WHICH HAVE BEEN IN COW TESTING ASSOCIATIONS FOR AT LEAST 8 YEARS.

The two scales are so chosen that one standard deviation on each occupies the same distance on the chart. Thus the slopes of the two lines show comparably their regression on the herd owner's experience in the cow testing association. Experience has a marked influence upon feed grade while the progress in production is slower, except during the first year.

#### *Influence of herd owner's experience*

Figure 1 shows that the average production of herds which have been in Cow Testing Associations for at least eight years gradually increases. The largest increase takes place from the first to the second year. Figure 1 also shows that the feeding improves rather steadily from year to year. Ten and three tenths per cent of the intra-herd variance disappears when the effect of yearly variations are eliminated. This 10.3 per cent is not very different in size from the 8.3 per cent of the intra-herd variance which is caused by differences in feeding. This may be largely accidental, but the similarity of the trends in Figure 1 indicates that the improvement in feeding as the owner's experience increases is an important factor in causing year to year variations within a herd.

#### *Influence of the length of the preceding dry period*

Investigations concerning the influence of the length of the dry period preceding lactation have usually shown that production is lowered when the dry period is extremely short. Most investigations have also shown

that very long dry periods tend to decrease the production in the following lactation. Thus there seems to be an optimum length of the dry period, usually given to be one to three months in length. Quite similar results were obtained in the present study but, excluding heifers, there were only 100 records which had no dry period at all. Slightly more than 1 per cent of the total variance disappeared when the effects of variation in dry period were removed. Apparently length of dry period is a minor cause of variation among these records.

#### *Influence of season of calving*

In the present data there are differences in production of cows calving at different seasons of the year. Cows calving during November to January produced 13.6 per cent more butterfat than cows calving in May to July, which is in close agreement with Cannon's (1) studies of more than 68,000 records from Iowa Cow Testing Associations. However, only 3 per cent of the total variance is due to differences in the season of calving. Thus although the season of the year has a significant influence upon production it is a minor cause of variation here.

#### *The relative importance of heredity and environment*

Gowen (8) has studied the relative importance of heredity and environment on milk production and butterfat percentage of Jersey Register of Merit cattle. He finds that the inheritance of the cow determines 50-70 per cent of the variation in milk production. Environment common to sisters accounts for 5 to 10 per cent and other factors peculiar to the cow herself account for 20-45 per cent. In the butterfat percentage the inheritance controls 75-85 per cent, the environment common to sisters has no apparent effect and the influences common only to the cow herself control 15-25 per cent. However in these calculations Gowen assumed that common environment contributed nothing to the correlation between daughter and dam. Such an assumption seems too extreme in view of the differences in herd management. Perhaps that goes far toward explaining the highness of the values he finds for the importance of heredity.

Wright (12) has calculated from Gowen's data from Holstein-Friesian and Guernsey cattle that milk production for these cows is determined about 75 per cent by heredity. Likewise Wright has calculated that the average correlation between the genotypes of the cows in a herd is about .20. This also interprets the differences between herd averages as much more genetic than environmental.

For cows tested under the variety of conditions found in Iowa Cow Testing Associations the statistics need not necessarily be the same as for cows on official test. There might be a wider range of environments (or a nar-

rower) in Cow Testing Association data. Likewise there might be a much wider or narrower range in the real hereditary productivity of the cows in the Cow Testing Association population.

In Table 3 it was shown that there is an intra-herd correlation between one record of a cow and another record of the same cow of .40. Now if heredity were the only thing which tended to make the records of the same cow alike, the portion of the intra-herd differences caused by heredity ( $h^2$ ) would be .40. Undoubtedly there are other factors which cause two records of a cow to be the same, as for instance intra-herd correlations between the environment to which the same cow has been exposed in the different years and intra-herd correlations between the cow's environment and her heredity. Such a correlation will exist if there is a tendency to feed individual cows according to production, but it has not been possible to measure it from the present data.

Hereditary differences between cows thus cause something less than 40 per cent of the variance found within a herd.

#### *The influence of common herd environment*

Possibly some herds are fed well because they produce well rather than the reverse, and therefore it may not be correct to say that the differences associated with differences in feeding are *caused* by differences in feeding. But with this qualification the feeding practices of the herds can be considered as a cause of variation between herds. However this accounts for only part of the differences between herds. The herds may differ in unrecorded ways in their feeding or in other important features of their management, such as times milked per day. On the other hand there doubtless are differences in the average intrinsic merit of the cows in different herds. The things which make herd mates resemble each other in productiveness may thus be partly hereditary and partly environmental.

The correlation of .33 between records of herd mates is a measure of the extent to which the combined genetic and non-genetic causes make them produce alike but tells nothing about how much of this is genetic and how much non-genetic. No satisfactory and simple answer to this was found. The writer's opinion is that differences between the average environment of different herds account for approximately three-fifths of the variance between herds. This figure is highly tentative, since it has not yet been possible to determine its value within narrow limits. The remaining two-fifths of the variance between herds can tentatively be regarded as due to hereditary differences in the average kind of cows in the different herds.

#### *Discussion of results*

The influence of common herd environment on correlations between relatives is a factor which should not be overlooked when aggregates of data from many herds are analyzed together.

The correlation between daughter and dam was .31 when the first available record of each was used and the data were analyzed together without regard to herd differences. The corresponding correlation within herds was .06 (based upon 683 dam-daughter pairs from 81 herds). Neither intra-herd assortative mating nor common environment can be very important in this intra-herd correlation. Doubling this correlation furnishes a rough estimate of the importance of heredity within the herd. However, only that fraction ( $g^2$ ) which can be expressed additively of the variance ( $h^2$ ) due to gene differences and combinations, enters into the daughter-dam correlation. This analysis therefore gives  $h^2g^2/2 = .06$  or  $h^2g^2 = .12$ . If dominance is the major cause of non-additive combination effects of genes, it is suggested by Fisher (2) that  $g^2$  is typically around  $\frac{2}{3}$  while Wright (11) for other reasons thinks that  $g^2$  is more typically around  $\frac{1}{5}$ . These two values would give values for  $h^2$  of .18 and .15 respectively. If non-additive interactions of non-allelomorphic genes (epistatic effects) are frequent and of considerable magnitude,  $g^2$  may be lower. Consequently  $h^2$  would take higher values than these, although it could not go above the .40 indicated by the intra-herd correlation between different records of the same cow.

In 246 cases (scattered through 68 herds) the dams had a record starting not more than 3 months from the time the daughter's record started and each dam also had an earlier record. When the first record was used for both daughter and dam, the total correlation was .32 while the correlation within herds was .10. For contemporary records of daughter and dam the total correlation was .40 and the correlation within herds was .27, showing a considerable effect of simultaneous environment. The total correlation between the two records of the dams was .41 while the correlation within herds was .25 for this. The correlation of .10 between the first available record of both daughter and dam gives, for values of  $g^2$  of  $\frac{2}{3}$  and  $\frac{4}{5}$ , values for  $h^2$  of .30 and .25 respectively. Lower values of  $g^2$ , which would occur if the effect of dominance is more than suggested by Wright and Fisher, or if epistatic effects are important, would give higher values of  $h^2$ .

Some other data,<sup>3</sup> collected in connection with the proved sire work in Iowa Cow Testing Associations, may be used for an independent estimate of the genetic part of the variance in butterfat production. These data consist of the records of 2,394 daughter-dam pairs used in proving 355 sires. An analysis of these records shows a total correlation of .408 and an intra-sire correlation of .178 between records of daughters and dams. These records were either lactation records or C. T. A. yearly records, corrected for age by the 70, 80, 90 per cent rule. Some of these records may have been made during the same year and there may have been other than genetic

<sup>3</sup> Kindly furnished by E. N. Shultz of the Dairy Husbandry Extension Service.

causes for this correlation. If genetic causes alone are responsible for this intra-sire correlation the genetic part of the variance ( $h^2g^2$ ) is at the most .36, which is slightly higher than the values found in the present study.

These correlations and the conditions under which they are obtained seem to make it likely that  $h^2$  within herds may be somewhere between .20 and .40. Until further studies have been made the value of  $h^2$  may be set at somewhere around one third to one fourth. This figure is not exact enough to be very satisfactory. Nevertheless it does explain and make reasonable a host of breeders' practical experiences which have hitherto seemed puzzling. It explains especially the many individual disappointments in selecting for high production in contrast with the favorable general experience with selection as a slow but fairly dependable average means of improvement. It is highly desirable that there be more studies to determine the value of  $h^2$  more closely. Especially would it be desirable to get more evidence about  $g^2$  on which the evidence here (only that involving correlations between relatives) is scanty. In measuring the immediate consequences of culling,  $h^2$  is important as expressing the portion which is real of the apparent difference between the productivity of those culled and those kept. But in selecting for breeding purposes,  $h^2g^2$  is more important since it is the fraction of superiority in the parents (as indicated by their records) which may be expected in their offspring.

The hereditary part of the herd differences (about 40 per cent as a very rough approximation) and the hereditary part of the intra-herd variance (one third) are not far from the same magnitude. Since both are approximations it may be well enough for practical purposes to combine them into a single value. A value of  $\frac{2}{3}$  would probably give a maximum estimate of the hereditary part of the variance among single records from many herds while a value of  $\frac{1}{3}$  would be more conservative.

The influence of year to year changes within a herd (probably mostly environmental but partly genetic, since some cows are culled and others added each year) accounts for 6 or 7 per cent of the total variance or roughly 10 per cent of the intra-herd variance. If this year-to-year variation were discounted by figuring production records within a herd as deviations from the yearly average, the resulting figures would doubtless come closer to representing hereditary differences in productivity, *i.e.*,  $h^2$  would be a little larger in them. However, yearly variations are not nearly so important in these Iowa records as they are said to be in the data used in Germany by von Patow and his associates (10) who seem to consider year-to-year variations as the major environmental cause of variation. Moreover the measurement of productivity as so many per cent above or below the herd average offers many practical difficulties in comparing cows which make records in different herds or in different years in the same herd, especially if the herds are small.

The estimate of the genetic part of the variance obtained from the present study is lower than the values given by Wright and by Gowen. Wright's unpublished computations (on Gowen's data from Holstein-Friesian and Guernsey Advanced Registry) seemed to indicate that nearly all the likeness between herd mates was genetic rather than due to common herd environment. This rested on the smallness of the difference between half sister correlations and non-sister herd-mate correlations as contrasted with the higher correlations between full sisters and between dam and daughter. Gowen's estimate of the magnitude of the environmental influence is what is called intra-herd year-to-year variations or "seasonal environment" in the present paper. If what seems to be the environmental part of the herd differences here (about 20 per cent) is subtracted from Gowen's figure for the influence of heredity, values are obtained which are only slightly higher than the values (one third) found in the present study. Gowen's estimate of the influence of environment (5 to 10 per cent) corresponds closely to the comparable value for intra-herd year-to-year changes in environment (about 10 per cent) found in the present study.

The correlations between records of the same cow reported by Gaines and Palfrey (4) and Gowen (7) would give values for the hereditary part of the variance which are only slightly different from the one found in the present study if environmental causes of differences between herds accounted for about 20 per cent of the total variance. Thus a value of about one third for the hereditary part of the variance ( $h^2$ ) should not seem surprisingly low.

It seems likely that the genetic part of the variance which enters into parent offspring correlations ( $h^2g^2$ ) is not far from one fourth. The practical consequence of such a value of  $h^2g^2$  would be that if the cows selected as parents for the next generation average 100 pounds of butterfat above the level of the breed or herd and the bulls are equally superior, then the offspring would average about 25 pounds above this same level. If the average interval between generations is 5 years this would correspond to an improvement of 5 pounds of butterfat per year. This rate of improvement could be increased if selection could be made more intense or if the genetic part of the variance could be increased, i.e., if other sources of variation can be controlled or taken into consideration by making proper corrections so that the breeder is no longer deceived by their effects.

While the results here are only approximate quantitatively, no reason is seen for thinking that they are biased either in the direction of largeness or smallness. Additional studies on suitable data with further refinements to verify and extend the conclusions of this study are to be desired. If and when such verifications are obtained, the conclusions can be the basis of improved practical procedure, especially in planning what is to be gained by this or that method of selection and in deciding how much attention to

pay to pedigree, to the animal's own production and to the production of its various progeny.

TABLE 4  
*Relative importance of causes of variation in butterfat production*

CAUSES OF VARIATION	PER CENT OF TOTAL VARIANCE	
Breed	2	
Herd		
Feeding policy of herd	12	
Other causes (genetic or environmental)	21	33
	—	
Cow (mostly genetic)	26	
Residual (year to year variations)		
Feeding variations within the herd	6	
Other year to year differences	1	
Length of dry period	1	
Season of calving	3	
Other factors	28	39
	—	
Total	100	

#### SUMMARY

The importance of different sources of variance is summarized in Table 4.

Practically all of the 2 per cent due to breed was found among the pure-bred cows, the grades showing very little breed difference.

Differences between cows (26 per cent) are mostly hereditary, but include the effects of any permanent change taking place in the cow before she starts giving milk and the effects of continually giving one cow better or poorer feeding and management than her herd mates.

Variation in butterfat production among these cows seems to have been determined about one third by differences in their heredity. The major source of uncertainty in this estimate is the question of what part heredity plays in the 21 per cent of the variance arising from herd differences not connected with recorded feeding practices.

The large amount (28 per cent) of unexplained variance indicates the considerable discrepancies still to be expected between a cow's record and her real producing ability, even after careful corrections have been made for age, feeding, dry period, calving season and the general level of the herd in which she is kept.

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# SUBSTANCES ADSORBED ON THE FAT GLOBULES IN CREAM AND THEIR RELATION TO CHURNING. IV. FACTORS INFLUENCING THE COMPOSITION OF THE ADSORPTION "MEMBRANE"\*

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## INTRODUCTION

Hattori (1), Palmer and Wiese (2), Wiese and Palmer (3) and Palmer and Lewis (4) have shown that the protein fraction of the natural fat globule "membrane" in milk is different chemically and physically from any of the known proteins of milk. Palmer and Samuelsson (5) and Palmer and Wiese (2) have demonstrated that the natural fat globule "membrane" also contains phospholipides which consist chiefly of a fraction resembling a lecithin.

The work of Wiese and Palmer (6) has shown that both "washed" cream and an artificial emulsion of butter-fat in buttermilk from sweet cream behave like normal cream in respect to churning, but that other artificial creams prepared with calcium caseinate, whey, blood globulin, phospholipide, or blood globulin plus phospholipide do not churn normally. It is thus demonstrated that the natural fat globule "membrane" is not formed when preparing artificial emulsions of butter-fat by means of the major constituents of milk plasma. Nevertheless, North and Sommer (7) erroneously assume that the natural interfacial material is concerned in the determination of the zeta potential between butter-fat and diluted skim milk.

The present paper deals with the isolation and comparison of the analyses of the fat globule "membrane" and its protein fraction from artificial emulsions of butter-fat in skim milk, buttermilk, whey, and casein solutions together with analyses of the natural fat globule "membrane" and its protein fraction.

## EXPERIMENTAL METHODS

All the creams, both natural and artificial, were washed by the method described by Wiese and Palmer (6).

The fat globule "membrane" materials were isolated as follows: The cream after washing was churned after cooling and tempering at a natural churning temperature. The free buttermilk combined with the buttermilk

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from the melted butter was supercentrifuged. The supercentrifuged butter-milk was dried at 40° C. under reduced pressure, the dried residue constituting the crude fat globule "membrane."

The protein portion of the "membrane" was the residue remaining after exhaustive extraction with absolute ethyl alcohol and ether.

The artificial emulsions of butter-fat were prepared by passing a mixture of melted, filtered butter-fat and the emulsifying solution at 38° through a Gaulin homogenizer five times at 200 pounds pressure. This treatment produced a "milk" in which the fat globules were similar in size to those found in natural milk.

The protein content of the "membrane" was determined by weighing the residue remaining after alcohol and ether extraction of a weighed sample of crude "membrane."

Aliquots of the alcohol-ether extract were ashed with  $MgNO_3$ , following the method of Wiese, Nair and Fleming (8) and the phospholipides calculated by multiplying the phosphorus content of the ash by 100 and dividing the result by 3.85 on the assumption that lecithin was the only phospholipide present. The assumption probably permits reasonably accurate comparisons between the different "membranes" studied.

The protein content of the washed cream was determined by weighing the residue left after treating a weighed sample of cream with dioxan<sup>1</sup> (ratio 1:7), filtering, extracting with ether and drying.

All phosphorus analyses were performed by the Fiske and Subbarow (9) method. Lipide phosphorus in cream was determined by the method of Wiese, Nair and Fleming (8).

Phosphatase activity of the creams was determined by the method of Kay and Graham (10).

The method reported by Cavett (11) was used for the Van Slyke nitrogen distribution of the "membrane" proteins.

#### DESCRIPTION OF THE CREAMS AND THEIR TREATMENT

*Natural creams.* *Cream 1.*—Two quarts of fresh cream were washed four times with distilled water. A sample of cream was taken after each

<sup>1</sup> Dioxan is a trade name for 1-4 dioxane (tetra-hydro-p-dioxin, or diethylene dioxide) manufactured by the Carbide, Carbon Chemicals Corporation, New York City. It is a good fat solvent and is miscible with water in all proportions. These facts make it useful in isolating the protein from cream. Preliminary experiments with mixtures of butter-fat, water and dioxan showed that the least amount of dioxan necessary to form a miscible mixture with 1 gram of a 50 per cent cream was 7 cc. and less dioxan was required for the higher fat creams. Since most washed creams contained over 50 per cent fat it was decided to use the ratio of 1 gram cream to 7 cc. dioxan in making the fat globule "membrane" protein analyses, and for creams with less than 50 per cent fat more dioxan was added until a miscible mixture was formed, when the samples were filtered. Although the dioxan dissolved the phospholipides and lipides, it was deemed necessary to extract the protein with ether in order to remove the last traces of these substances.

washing and saved for total phosphorus, lipid phosphorus, and protein analyses. The fat globule "membrane" was not isolated from this cream.

*Cream 2.*—One gallon of fresh cream was washed with distilled water. One quart was taken out after the fourth, sixth, eighth and tenth washings, and they were used for isolation of the fat globule "membrane." The total phosphorus, lipid phosphorus, and protein were determined in the creams washed four, eight, and ten times. The cream washed six times was lost.

There was not enough "membrane" protein isolated from the samples of creams that were washed four and eight times respectively to determine the Van Slyke nitrogen distribution; however, enough "membrane" protein was obtained for this determination from the sample of cream washed ten times.

*Cream 3.*—One gallon of fresh cream was washed four times with distilled water. The fat globule "membrane" was then isolated and analyzed.

*Cream 4.*—One gallon of fresh cream was washed six times with distilled water, and the "membrane" isolated and analyzed. A sample of the original cream and samples from the first, fourth, and sixth washings were saved for phosphatase determinations.

*Cream 5.*—Fresh cream was washed with distilled water twenty-four times and samples withdrawn at the fourth, eighth, twelfth and twenty-fourth washing. The samples, together with one of the original cream, were preserved with formaldehyde, adding about 1 part HCHO per 2,500 parts cream.

*Cream 6.*—Fresh cream was washed eight times and samples withdrawn for analysis after the fourth and last washings. The samples, together with a sample of the unwashed cream, were preserved with formaldehyde (1:2,500).

*Cream 7.*—This was a repetition of cream 6 except the samples were not preserved with formaldehyde.

*Cream 8.*—Two quarts of fresh cream were washed four times with distilled water, then four times with sweet rennet whey, and finally four times with water. Sweet rennet whey was prepared by adding one and one-half times the required amount of rennet to fresh skim milk, cutting the curd with cheese knives, cooking it slightly, and finally drawing off the whey. This whey was immediately centrifuged to remove the casein particles, cooled, and stored in a refrigerator until used the next day. Before the whey was used for washing the cream, it was warmed to 38°–43°.

Samples of cream were saved from the following washings; fourth whey washing, and the first, second, third and fourth water washings following the whey washing. These samples were analyzed for total phosphorus, lipid phosphorus, and protein. The sample of cream which had been washed twelve times (four times by water, four times by whey, and again four times by water) was used for isolation of the fat globule "membrane."

*Artificial creams. Cream 9. Whey cream.*—The sweet rennet whey used

in making this cream was prepared by the same method as that described for the whey used to wash cream 8. Butter-fat was obtained by filtering melted butter. The emulsion of butter-fat in whey was made by the method described under experimental methods.

The whey cream was washed twice without difficulty, but during the third washing, the cream clogged the centrifuge bowl and had to be scraped out and redispersed in water. This redispersed cream constituted the third washing and was used for isolation of the "membrane." No attempt was made to wash the cream more than three times.

*Cream 10. Skim milk cream.*—The skim milk used for the preparation of this cream was obtained from the separation of fresh whole milk. It was used immediately for making the butter-fat emulsion.

The cream thus obtained was washed twice without trouble, but both the third and fourth washings had to be scraped out of the centrifuge bowl and redispersed in water. Samples were taken from the original cream and from the first, second, third and fourth washings, and were used to determine their total phosphorus, lipide phosphorus and protein contents. A sample of the fourth washing was also used for isolation of the "membrane" material.

*Cream 11. Buttermilk cream.*—The buttermilk used for the preparation of this cream was obtained from the churning of fresh unpasteurized sweet cream. It was then supercentrifuged to remove all traces of unchurned fat globules. Butter-fat was obtained from filtered melted butter.

The buttermilk cream was washed three times but the third washing of the cream had to be scraped from the centrifuge bowl and redispersed in water. No attempt was made to wash the cream more than three times. The redispersion of the cream from the centrifuge bowl constituted the third washing which was analyzed for total phosphorus and lipide phosphorus, and also used for the isolation of the fat globule "membrane" protein. Samples of the original cream and of the first and second washings were analyzed for total and lipide phosphorus.

*Cream 12. Buttermilk cream.*—Buttermilk used for this experiment was prepared by churning two gallons of unpasteurized cream in a metal barrel churn. The buttermilk was then centrifuged to remove unchurned fat globules and particles of butter-fat. The method for preparing this cream was not the same as that used for making the other artificial creams. Instead of emulsifying butter-fat into buttermilk to produce a four per cent milk, sufficient butter-fat was used to make a cream having thirty-five per cent fat. This cream was washed twice without much difficulty, but the separation of the third washing clogged the centrifuge bowl. The cream from the bowl was redispersed in distilled water and was used for the isolation of the fat globule "membrane."

*Cream 13. Casein cream.*—The casein was prepared from skim milk by the Van Slyke and Baker (12) method modified by Van Slyke (13). A solu-

tion containing 2.5 per cent casein was made by dissolving the casein in saturated lime water and titrating this solution to a pH 6.6 with one per cent  $\text{H}_3\text{PO}_4$ . This casein solution was mixed with enough butter-fat to form a thirty-five per cent cream, and the mixture emulsified.

The casein cream could not be washed with water as it clogged the centrifuge bowl during the first washing.

#### EXPERIMENTAL RESULTS

*The effect of washing cream with water upon its lipid phosphorus, total phosphorus, and protein content.* The results of these experiments are shown in Table 1. After the third washing the decrease in the above mentioned constituents in cream 1 was very small. There was further gradual decrease of less magnitude in lipid phosphorus and total phosphorus after the fourth washing, which continued until the emulsion broke. This fact is illustrated in the experiments with cream 11. However, after the twenty-fourth washing the cream that remained still contained the fat globules with the "membrane" intact, but weakened. The "membrane" material is held very tightly to the fat globules.

The lipid phosphorus analyses 1, 2 and 3 in Table I were made on the same sample of cream at intervals of about a week or more. These tests were made to investigate the possible deterioration of these "membrane" constituents upon standing. There was a decrease in lipid phosphorus in creams 2 and 5, but none in creams 6 and 7. Since formaldehyde was added to cream 6 but not to cream 7, these contradictory results leave the question of "membrane" lipid deterioration open to further investigation; they suggest that the "membrane" may at times carry a lecithinase.

The washed artificial creams contained very little lipid phosphorus, showing that the "membrane" was not typical of that found in natural creams.

Cream 8 washed four times with water, four times with whey, and finally four times with water, had a final lipid phosphorus content lower than that observed for cream 5 washed twelve times with water. One of the major effects of washing with whey was to decrease the lipid phosphorus content of the "membrane."

All the natural creams withstood washing with water even up to twelve times as is shown by cream 5. This cream began to break after the twelfth washing but some cream was left even after twenty-four washings.

The artificial creams could not be washed more than three times at the most, which is further evidence that their "membranes" were not typical of natural cream.

*The effect of washing cream on its phosphatase activity.*—The effect of washing cream on its phosphatase activity is shown in Table 2. After washing six times with distilled water the cream still contained more than 50 per

cent of its phosphatase activity. During the churning of the washed cream the phosphatase evidently goes into the buttermilk as is shown by the high enzyme content of the buttermilk from melted butter. This shows definitely that part of this enzyme in cream is a constituent of the fat globule "membrane" and can not be washed away with water, and substantiates the conclusions of Kay and Graham (10) based on the phosphatase activity of various fractions of milk, whole milk, skim milk, cream, butter, buttermilk.

*Composition of the fat globule "membrane."*—Table 3 gives the analyses of the crude fat globule "membranes" isolated from washed natural cream. The range in composition of the fat globule "membranes" is as follows: Protein 21.98 to 43.96 per cent, phospholipides 13.00 to 26.00 per cent, and non-phospholipide ether-extractable fraction 56.04–78.02 per cent. The "membrane" from artificial creams contained only traces of phospholipides, but contained 44.66 to 55.25 per cent protein and 44.75 to 55.34 per cent non-phospholipide ether-extractable material.

*Composition of the fat globule "membrane" protein.*—Table 4 contains the results of all the "membrane" protein analyses performed compared with similar analyses of the "membrane" protein previously published from this laboratory.

*Comparison of the natural fat globule "membrane" proteins with each other.*—There are small significant variations in total nitrogen, Van Slyke nitrogen distribution, and phosphorus content among the different natural cream "membrane" proteins, but as a group they compare favorably with those isolated by Hattori (1) and Wiese and Palmer (3). It is evident from these analyses that the "membrane" protein is not one of the common milk proteins. The variations in analyses of the "membrane" protein which were found in this study indicate that the methods employed for its isolation do not result in a "pure" protein but rather a protein contaminated by varying although minor amounts of the other proteins of milk.

*Comparison of the natural fat globule "membrane" protein with those isolated from artificial creams.*—The Van Slyke nitrogen distribution and phosphorus analyses show definitely that the fat globule "membrane" proteins of the artificial creams, are not the same as those isolated from natural creams.

*Comparison of the "membrane" proteins among the individual artificial creams.*—The "membrane" proteins from creams 11 and 12 (buttermilk creams) are very similar to each other in all respects, but differ from the "membrane" proteins isolated from cream 9 (whey cream) and cream 10 (skim milk cream). The "membrane" protein from cream 9 (whey cream), resembles in its Van Slyke nitrogen distribution a mixture of lactalbumin and globulin, and that from cream 10 (skim milk) a mixture of casein and lactalbumin.

*The total nitrogen content of the "membrane" proteins.*—All the "mem-

brane" proteins, both from natural and artificial creams, have a low nitrogen value, much lower than observed for pure proteins. The cause for this low nitrogen content is not known. Attempts to detect carbohydrate or sterol as the prosthetic group of the natural "membrane" protein have not proved successful; also the low nitrogen is not explained by a high mineral content.

#### DISCUSSION

Those substances in milk plasma which lower the tension of a butter-fat-water interface would be expected to concentrate at the surface of the fat globules. However, in milk it is evident that the fat globules are wholly or partially surrounded by a special group of substances whose origin has not yet been explained and whose presence may or may not be due wholly to their colloidal properties. The surface active substances occurring in major concentration in milk plasma evidently constitute the outer layers of the fat globule surfaces if indeed they are normally concentrated there at all. For when cream is washed by dilution with water the surface active substances of milk plasma are readily removed leaving essentially only the special, probably highly oriented material as the natural "membrane" layer around the fat globules.

This special "membrane" material has been shown in this study to be composed of protein, phospholipides, and other extractable non-phospholipides. This is in agreement with the earlier work of Palmer and Samuelson (5) and Palmer and Wiese (2). The significance of the ether-extractable non-phospholipide material which composes over 50 per cent of the "membrane" can not be stated at the present time. It is also found in the fat globule "membranes" of the artificial creams. Perhaps the oriented molecules of "membrane" material are partly in the fat phase and are attached to certain of the fat molecules with almost chemical affinity; when the fat globules coalesce during the churning process to become the continuous phase the "membrane" molecules possibly continue to hold onto this fat which can only be removed by fat solvents.

It was found in this study that the "membrane" composition is variable from one natural cream to another in respect to the protein and phospholipide content, and also in respect to the Van Slyke nitrogen distribution of the protein fraction although the latter variation is only slightly significant. The cause of these variations remains to be determined. The mechanical methods employed are no doubt responsible in part but the possibility of certain variations being of biological origin, *i.e.*, natural variations, cannot be overlooked.

Repeated washing with water slowly wears away the "membrane" around the fat globules in natural cream, but it takes more than twelve washings before the emulsion begins to break, and even after twenty-four

washings the cream remaining still contains fat globules with their "membrane" intact.

Washing, water-washed cream, with whey in some way affects the lipide phosphorus fraction so that this can be washed away by further water washing much more readily than when water alone is used. This is one of the most surprising results of the present study and shows that the lipide fraction cannot be regarded as a chemical component of the "membrane" protein molecules.

The "membrane" materials isolated from artificial creams made by emulsifying butter-fat in either whey, skim milk or buttermilk were distinctly different from those isolated from natural creams. In the first place these artificial creams could not be washed as extensively as could the natural creams. Furthermore the "membrane" around the fat globules in artificial creams contained little or no phospholipides, the largest amount occurring in the "membrane" from the buttermilk cream. The proteins from the fat globule "membranes" of the artificial creams were found to be distinctly different from the natural "membrane" proteins in respect to Van Slyke nitrogen distribution and phosphorus analyses. Even the "membrane" isolated from buttermilk creams was not identical with the natural "membrane" materials, although buttermilk is known to contain these materials. This seems contrary to the experience of Wiese and Palmer (6) who found that artificial buttermilk cream churned normally. The explanation may lie in the fact that the unwashed buttermilk cream is as rich in phospholipides as many samples of washed natural cream (See Table 1). It is possible, therefore, that a small but significant part of the "membrane" in this case is the natural "membrane" material occurring in the buttermilk, the amount being sufficient to account for its normal churning behavior.

These results show that the recent work of North and Sommer (7) may be criticized because they assume that by streaming diluted skim milk through a butter-fat capillary they are dealing with the same "membrane" that is found around natural fat globules in milk. The emulsions of butter-fat in skim milk do not produce typical "membrane" material around the fat globules as evidenced by the chemical analyses reported in this paper and the other abnormal behavior of artificial skim milk creams.

The Van Slyke nitrogen distribution of the fat globule "membrane" proteins of natural cream resembles more the nitrogen distribution of the "membrane" proteins isolated by Hattori (1) and Wiese and Palmer (2) than that of the "membrane" proteins isolated from artificial creams or that of the ordinary milk proteins. The "membrane" proteins from the artificial creams resemble mixtures of known milk proteins in regard to their Van Slyke nitrogen distributions.

This dissimilarity between the fat globule "membrane" materials of artificial creams and the natural "membrane" material raises the question

of the origin of the natural fat globule "membrane." It appears possible that the "membrane" may be formed before the fat globules become a part of the milk or the fat globules may be secreted before the milk plasma is completely formed in which case the "membrane" materials could be considered, in part at least, as precursors of plasma materials.

The nitrogen content of fat globule "membrane" proteins of both the artificial and natural creams is lower than that observed for known proteins. We have no explanation for these low total nitrogen values at the present time, but we do know they are not due to cholesterol, carbohydrate or to the presence of large amounts of inorganic materials since analyses have been made for these substances.

Chrzaszcz and Goralowna (14) have shown that diastase, reductase and catalase are present in higher concentration in cream than in milk; Wieland and Macrae (15) have shown this to be true also of dehydrogenases; Tayama (16) has demonstrated that zanthine oxidase is present on washed fat globules; and Kay and Graham (10) have shown that phosphatase is closely associated with the fat globules in milk. Our study has demonstrated conclusively that phosphatase is a definite constituent of the natural fat globule "membrane." The various enzymes mentioned and others for which tests have not been made possibly play an important part in the keeping qualities of the fat-rich fractions of milk. This is suggested by the fact that in the preservation of the samples from the cream washing experiments certain samples showed a deterioration of the phospholipide fraction, which was probably due to enzyme action, possibly a lecithinase.

#### CONCLUSIONS

1. The percentage of protein and phospholipides in the fat globule "membrane" from various samples of cream is not constant but is essentially constant for any one sample of cream after the fourth washing with distilled water, at least through the tenth washing.

2. The adsorption "membrane" found on butter-fat globules emulsified in sweet rennet-whey, skim milk, or buttermilk does not have the same composition as the natural fat globule "membrane" of milk in its percentage and proportion of protein and phospholipides.

3. The Van Slyke nitrogen distribution of the fat globules "membrane" proteins from artificial creams prepared by emulsifying butter-fat in whey, skim milk or buttermilk, is not the same as that of the natural fat globule "membrane" proteins.

4. A large part of the phosphatase activity of natural cream is in the fat globule "membrane" material and is not removed by water washing.

5. In the synthesis of milk the natural fat globule "membrane" is not derived from the milk plasma.

6. The fat globule "membrane" proteins of natural cream and of artificial creams made by emulsifying butter-fat in skim milk, buttermilk or whey appear to contain a prosthetic group, so far not identified, which causes the nitrogen content to be abnormally low as compared with other known proteins of similar complexity.

TABLE 1  
*The effect of washing cream on its lipid phosphorus, total phosphorus and protein content*

CREAM	PHOSPHORUS PER 100 GRAMS FAT			Total P	PROTEIN PER 100 GRAMS FAT
	Lipide <sup>1</sup> P				
	1	2	3		
	mgms.	mgms.	mgms.	mgms.	grams
Natural creams (washed with water)					
Cream 1					
Original cream	17.3			188.3	11.4
After first washing	12.9			39.7	1.66
After second washing	9.3			18.6	0.73
After third washing	8.7			15.3	0.61
After fourth washing	7.2			13.8	0.59
Cream 2					
After fourth washing	8.3			11.8	0.45
After eighth washing	10.6		5.8	12.2	0.46
After tenth washing	8.5			11.2	0.45
Cream 5					
Original cream	15.6			128.8	
After fourth washing	13.6	10.2	8.6	18.2	0.69
After eighth washing	11.1		8.7	16.4	0.61
After twelfth washing	7.1	7.3		16.5	0.54
After twenty-fourth washing	5.4			10.4	0.46
Cream 6					
Original cream	20.2	17.8			
After fourth washing	13.3	15.0	14.0	21.3	
After eighth washing	11.3	12.5	11.2	16.6	0.71
Cream 7					
Original cream	18.8	16.5			
After fourth washing	13.6	12.3		18.2	
After eighth washing	10.3		10.9	17.9	0.68
Cream 8 <sup>2</sup>					
After fourth whey washing	10.3			137.2	
After first water washing <sup>3</sup>	7.2			26.5	
After second water washing	4.3			12.2	
After third water washing	4.1			10.3	
After fourth water washing	3.7			9.3	0.36
Artificial creams (washed with water)					
Cream 9 (whey cream)					
Original cream	3.39				
After third washing	0.66			2.8	0.57
Cream 10 (skim milk cream)					
Original cream	0.68			189.0	
After fourth washing	0.14			5.5	0.65
Cream 11 (buttermilk cream)					
Original cream	7.63			191.6	
After first washing	1.50			37.0	
After third washing	1.20			25.8	

<sup>1</sup> Analyses 1, 2 and 3 were made on the same sample at about week intervals.

<sup>2</sup> Washed with water 4 times, whey 4 times, then water 4 times.

<sup>3</sup> Following the whey washings.

TABLE 2

*Phosphatase activity of cream washed with water and of the buttermilk from the cream washed six times*

	PHOSPHATASE UNITS <sup>1</sup> IN CREAMS	PHOSPHATASE UNITS <sup>1</sup> PER GRAM OF FAT
Cream 4		
Original cream	3.00 per gram	6.84
After first washing	1.99 " "	5.73
After fourth washing	1.69 " "	4.94
After sixth washing	1.52 " "	4.22
	1.43 per cc. }	
	4.18 per cc. }	3.41

<sup>1</sup> Kay and Graham units (10).

TABLE 3

*Composition of the fat globule "membranes" isolated from natural and artificial creams*

	PROTEIN	ALCOHOL ETHER EXTRACT		
		Phospho- lipide <sup>1</sup>	Non phos- pholipide	Total
Normal creams	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Cream 2				
Washed four times with water	43.96	17.53	38.52	56.04
Washed eight times with water	40.12	19.46	40.42	59.88
Washed ten times with water	39.13	21.96	38.91	60.87
Cream 3				
Washed four times with water	29.13	26.00	44.87	70.87
Cream 4				
Washed six times with water	21.98			78.02
Cream 6				
Washed eight times with water	36.30	13.00	50.70	63.70
Cream 7				
Washed eight times with water	33.29	22.51	43.80	66.71
Cream 8				
Washed four times with water, four with whey, and four with water	34.76			65.24
Artificial creams				
Cream 9 (whey cream)				
Washed three times with water	54.43			45.57
Cream 10 (skim milk cream)				
Washed four times with water	44.66			55.34
Cream 11 (buttermilk cream)				
Washed three times with water	55.25			44.75
Cream 12 (buttermilk cream)				
Washed three times with water	52.25			47.75

<sup>1</sup> Calculated from the percentage total phosphorus in alcohol and ether extract using factor 3.85% P in phospholipide.

TABLE 4  
Average<sup>1</sup> analyses of fat globule "membrane" proteins

	CREAM 2 <sup>2</sup>	CREAM 3 <sup>3</sup>	CREAM 4 <sup>4</sup>	CREAM 5 <sup>5</sup>	CREAM 9 <sup>6</sup>	CREAM 10 <sup>7</sup>	CREAM 11 <sup>8</sup>	CREAM 12 <sup>9</sup>	PREVIOUS ANALYSES <sup>10</sup>
Van Slyke Nitrogen Distribution per cent									
Ammonia-N	11.94	10.16	11.58	11.68	13.19	13.22	11.31	11.98	6.54
Humin-N	4.69	3.73	4.21	4.58	3.78	3.19	2.74	2.48	3.86
Arginine-N	13.59	10.77	12.16	11.27	10.06	9.64	7.36	7.43	15.82
Cystine-N	0.27	0.48	0.675	0.70	0.73	0.46	0.37	0.49	0.72
Histidine-N	1.00	5.64	3.63	3.67	3.10	3.60	2.41	5.02	1.69
Lysine-N	11.36	8.60	10.27	8.53	12.19	11.68	13.08	11.53	11.49
Basic NH <sub>2</sub> -N	13.42	13.64	15.20	13.27	16.48	15.75	16.10	15.55	16.73
Total basic-N	26.23	25.49	26.74	24.18	26.09	25.38	23.23	24.47	29.71
Non basic NH <sub>2</sub> -N	55.69	57.51	55.79	57.83	54.20	53.96	57.73	55.56	56.50
Non basic non NH <sub>2</sub> -N	4.20	3.84	3.68	3.20	3.99	4.57	5.51	7.08	3.51
Total non basic-N	59.89	61.35	59.47	61.03	58.19	58.63	63.24	62.64	60.01
Total recovery	102.76	100.73	102.00	101.48	101.26	100.43	100.53	101.58	100.12
Total N	13.07	13.48	13.45	12.74	12.35	13.31	13.44	13.75	12.22
Phosphorus	0.492	0.489	0.388	0.446	0.350	0.877	1.290	1.350	0.33
Calcium	-	0.484	0.244	-	-	-	-	-	-
Ash	-	2.83	2.00	-	-	-	-	-	-

<sup>1</sup> Average of two analyses agreeing within the limits permitted for these determinations (Cavett (1932)).

<sup>2</sup> Natural cream washed ten times with water.

<sup>3</sup> Natural cream washed four times with water.

<sup>4</sup> Natural cream washed six times with water.

<sup>5</sup> Natural cream washed four times with water, four times with whey and then four times with water.

<sup>6</sup> Artificial whey cream washed three times with water.

<sup>7</sup> Artificial skim milk cream washed four times with water.

<sup>8</sup> Artificial buttermilk cream washed three times with water.

<sup>9</sup> Artificial buttermilk cream washed three times with water.

<sup>10</sup> Average of analyses made by Wiese and Palmer (3).

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# American Dairy Science Association Announcements

## ANNUAL MEETING, JUNE 16-19, 1936

The annual meeting of the American Dairy Science Association will be held at the Pennsylvania State College June 16-19, 1936. The Program Committee will welcome suggestions from the membership concerning new features or changes from the type of program that we have followed in past meetings. Suggestions may be sent directly to S. I. Bechdel, chairman of the Program Committee, or to any of the general officers of the Association or to the officers of the Sections.

## NEW OFFICERS

Results of the election of officers are not generally mailed to members but the directory page as carried in the Journal is corrected as rapidly as changes are brought to the attention of the editor. It is believed that list now contains all of the newly elected officers, most of whom took office on October first.

## JOURNAL INDEX

The system of indexing the Journal has been changed in the present volume to care for the increased size of the Journal.

The complete papers and abstracts of papers given at the annual meeting have been grouped together and each article appears at least three times in the subject index. The author index has been separated from the subject index. Since the editor has planned on following this same style of index and similar main index headings, comments to improve it would be appreciated.

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CONTENTS

*Corrections for article by*

H. A. LASBY and L. S. PALMER

RECEIVED



# NUTRITIONAL ANEMIA, CALCIUM PHOSPHORUS AND NITROGEN BALANCE AND BONE COMPOSITION OF RATS FED RAW VERSUS PASTEURIZED MILK

H. A. LASBY and L. S. PALMER

Due to a number of unfortunate errors in the calculation of the chemical data and statistical significance of the results the following corrections are required in the paper bearing the above title in this Journal Vol. 18, No. 3, March 1935, pages 181 to 192.

On page 183 last paragraph ending on page 184 read the following in place of the entire paragraph: Using 'Student's' formula for  $t$  as applied to small samples, calculating the probability ( $P$ ) as  $(1-\alpha)$  from 'Student's' tables<sup>1</sup> for  $1/2 (1 + \alpha)$  and selecting a 5 per cent level for significance the data in Table 1 show that the differences for each successive weekly interval are not significant.

On page 185, line 1 read "third week" in place of "second week."

On page 186, lines 3, 4, 5 and 6 read the following: However, Table 2 shows that the difference was significant only in the third week, and, being only slightly significant as well as being followed by differences which were not significant, cannot be given much consideration.

On page 188 omit the last sentence beginning, "The slight differences—."

On page 190, lines 16 and 17 read the following in place of the entire sentence: *Results.* Results of bone analyses of ten of the eleven pairs of rats are presented in Table 5, the data from pair No. 15 being omitted from the calculations because the animals remained in the experiment only 17 days.

On page 190, line 19, read 0.0076 for 0.054; line 21, read "significant" for "border line"; line 25, read  $+0.6259$  for  $+0.7638$ ; lines 24, 25 and 26 read in place of the sentence beginning, "A correlation—" the following: A correlation coefficient of  $+0.6259$  was obtained for the bone ash of the rats fed the raw milk and a correlation coefficient of  $+0.4043$  for the bone ash of the rats fed pasteurized milk, the value for the raw milk fed animals having a border line significance for the number of samples.

On page 191, line 4, read  $+0.5088$  for  $+0.6238$  and read  $+0.3690$  for  $+0.2493$ ; line 6, read "statistically" for "probably not." Add the following: The biological significance of the result is rendered improbable by the fact that similar calculations for the calcium and phosphorus content of the same bones showed probability values for significance of 0.3492 and 0.3839, respectively. Line 28, read: 1. The calcium retentions were slightly greater on the raw milk but the difference is not statistically significant; line 29, read: 2. The phos-

<sup>1</sup> Metron 5:26-29. 1925.

phorus retentions were the same on the two diets; line 32 read "raw" for "pasteurized."

On page 192, lines 6 and 7, read in place of the sentence beginning. "The difference—," the following: The difference is statistically but not biologically significant and is not explained on the basis of the better growth of the rats receiving the pasteurized milk; lines 10, 11 and 12, omit the sentence beginning "The ash—."

In addition to the above corrections substitute the following tables, 1, 2, 3, 4 and 5, for the corresponding tables on pages 184, 185, 189, 190 and 191, respectively.

TABLE 1

CALCULATIONS FROM HEMOGLOBIN			CALCULATIONS FROM BODY WEIGHT	
<i>Week</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
0	0.5619	0.5850	0.2552	0.8042
1	0.1996	.8452	0.2443	.8114
2	0.2012	.8358	0.1218	.9052
3	0.2923	.7752	0.4542	.6582
4	0.1075	.9166	0.1649	.8724
5	1.1926	.2608	0.1848	.8572
6	0.2547	.8056	0.0498	.9614
7	0.9271	.3902	0.8960	.4048
8	1.3656	.2214	0.7519	.4812

TABLE 2

CALCULATIONS FROM HEMOGLOBIN			CALCULATIONS FROM BODY WEIGHT	
<i>Week</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
0	0.1123	0.9118	0.3153	0.7564
1	0.4161	.8624	0.8945	.3830
2	1.3814	.0920	0.6890	.4998
3	2.2815	.0366	2.2127	.0402
4	2.8422	.0120	1.0197	.3234
5	5.5720	.0002	1.1952	.2596
6	6.5607	.0000	2.0302	.0700
7	3.6187	.0154	2.0256	.0894

TABLE 3

PAIR NO.	BAT	DIET	Ca		TOTAL Ca ex-cretion	Ca		Ca intake retained	Ca intake per cent	P		TOTAL P excretion	P		P intake retained	P intake per cent	N		TOTAL N excretion	N intake retained	N intake per cent	
			intake	mgm.		balance	mgm.			intake	mgm.		balance	mgm.			intake	mgm.				balance
1.	1728	1. raw	556.3	344.6		211.7	38.06	439.0		188.1	42.84	250.9	2887.2	2146.1	741.1	25.67						
	1730	1. past.	447.1	394.6		52.5	11.74	413.1		145.4	35.20	267.7	145.4	1821.1	741.5	28.93						
	1729	1. raw	383.8	263.3		120.5	31.39	302.9		90.8	29.97	212.1	2953.6	1536.5	455.7	22.87						
2.	1731	2. past.	624.5	489.0		153.5	21.71	503.4		216.2	42.95	287.2	216.2	1992.2	483.0	16.35						
	1731	1. past.	315.4	245.3		70.1	22.20	291.3		98.9	33.96	190.3	1807.5	1389.7	417.8	23.12						
	1733	2. raw	632.8	478.8		154.0	24.35	520.0		239.7	46.10	280.3	2901.6	2268.7	632.9	21.81						
3.	1733	1. raw	525.6	300.2		225.4	42.88	414.7		141.5	34.13	273.2	141.5	1754.5	973.4	35.68						
	2. past.	645.2	379.1		266.1	41.25	456.7		223.0	233.7	51.17	223.0	3213.0	1960.9	1252.1	38.97						
	1735	3. raw	610.3	472.5		137.8	22.58	464.8		194.3	41.80	270.5	194.3	2420.0	510.1	17.41						
4.	1735	1. past.	452.1	297.1		155.0	34.28	417.7		256.4	38.61	256.4	161.3	2591.2	1833.0	758.2	29.26					
	2. raw	625.8	364.1		261.7	41.82	456.7		234.4	222.3	48.67	234.4	222.3	3108.0	747.4	24.05						
	3. past.	620.1	397.9		222.2	35.83	458.5		244.9	213.6	46.59	244.9	213.6	2985.3	2415.1	570.2	19.10					
4.	1766	1. raw	625.5	393.9		231.6	37.02	413.9		239.8	42.06	239.8	174.1	2391.5	1849.6	541.9	22.66					
	2. past.	604.5	488.0		116.5	19.27	465.2		276.1	189.1	40.66	276.1	189.1	643.9								
	1767"	3. raw	603.8	490.4		113.4	18.77	474.6		264.8	44.20	264.8	209.8	1723.5								
5.	1765	1. past.	576.3	367.5		308.7	36.23	398.7		157.3	39.46	241.4	157.3	2367.4								
	2. raw	624.5	436.3		188.2	30.13	442.7		253.0	189.7	42.86	253.0	189.7									
	3. past.	622.6	448.3		174.3	28.00	476.6		254.7	221.9	46.56	254.7	221.9									
5.	1765	1. raw	581.2	333.4		247.8	42.64	384.6		159.9	41.58	224.7	159.9	2593.5								
	2. past.	612.3	421.9		190.4	31.09	471.2		269.5	201.7	42.81	269.5	201.7	2756.8								
	3. raw	639.9	479.5		160.4	25.07	502.9		284.2	218.7	43.49	284.2	218.7	2307.1								
5.	1764"	1. past.	561.6	319.5		242.1	43.11	388.6		191.4	50.76	319.5	191.4	1672.5								
	2. raw	628.4	464.7		163.7	26.06	445.6		277.0	168.6	37.83	277.0	168.6	2153.9								
	3. past.	643.5	461.1		182.4	28.34	492.7		294.7	198.0	40.18	294.7	198.0	2309.3								
6.	1767	1. raw	498.4	337.0		161.4	32.39	329.8		117.8	35.71	212.0	117.8	1388.9								
	2. past.	483.4	346.3		137.1	28.36	372.0		229.6	142.4	38.28	229.6	142.4	1430.7								
	3. raw	538.5	391.2		147.3	27.36	423.3		270.6	182.7	43.16	270.6	182.7	1753.8								
6.	1769	1. past.	382.9	280.3		102.6	26.80	264.9		45.5	17.16	219.4	45.5	1314.4								
	2. raw	480.2	352.2		128.0	26.66	340.5		225.6	114.9	33.74	225.6	114.9	258.4								
	3. past	547.0	409.4		137.6	25.15	418.8		252.2	166.6	39.77	252.2	166.6	1913.0								

TABLE 4

ELEMENT	RETENTION ON RAW MILK	RETENTION ON PASTEURIZED MILK	P
	<i>per cent</i>	<i>per cent</i>	
Calcium . . . . .	31.15	28.89	0.4608
Phosphorus . . . . .	40.54	40.27	.9138
Nitrogen . . . . .	24.49	23.55	.7506

TABLE 5

MILK	BONE COMPOSITION			TOTAL LENGTH FEMUR PLUS TIBIA
	Total Ash	Ca	P	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>mm.</i>
Raw . . . . .	59.29	21.97	9.36	5.353
Pasteurized . . . . .	56.56	21.53	9.12	5.536





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